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ORIGINAL ARTICLES

FRACTIONATION OF SOIL PHOSPHORUS

III. THE ORGANIC PHOSPHORUS FRACTION

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(Received for publication on 14 April 1944)

In the two previous communications of this series [Ghani, 1943, 1 ; Ghani and Aleem, 1943] a method was outlined for the fractionation of soil phosphorus, and the chemical nature of the inorganic phosphorus fractions was elucidated. The organic phosphorus fraction was determined in the alkali extracts of soils, pretreated with N/2 acetic acid, by the bromine oxidation method of Dean [1938] modified by Ghani [1942]. The condition of alkali extraction with respect to the kind of alkali and its concentration, soil-solvent ratio, time and temperature of extraction and the technique of extraction were all defined there. The organic phosphorus determined in the alkali extracts was taken to be the total organic phosphorus of the soil. An attempt has been made here to determine the organic phosphorus of the soil by an independent method with a view to testing the validity of the fractionation method, so far as the determination of the organic phosphorus is concerned. The work was carried out at the Rothamsted Experimental Station, England, as a continuation of the work already reported by the author [Ghani, 1943, 1].

Determination of organic phosphorus by ignition method

It was observed by the earlier workers on soil phosphates that ignition of the soil increased solubility of soil phosphorus in dilute mineral acids. It was assumed that the effect of ignition was to destroy the organic phosphorus compounds and thus render the phosphates soluble in acid, without affecting the solubility of inorganic phosphates. A method for determination of organic phosphorus in soil was based on this assumption. A portion of the soil was ignited, extracted with mineral acid of certain strength and phosphoric acid estimated in the extract. From this was subtracted the amount of phosphorus extracted from the ignited soil by the same solvent under identical conditions of extraction and the result was taken to represent the organic phosphorus of the soil. Stewart [1910] found that ignition method gave results higher than the ammonia extraction method and Fraps [1911] contended that it includes some of the mineral phosphates which are rendered more soluble in acid by ignition. Peterson [1912] refuted the criticism of Fraps and showed that the solubility of the mineral phosphates of the soil did not increase by heating. On the face of such conflicting evidence, no further use of the method appears to have been made till comparatively recently.

Odynisky [1936] adopted the ignition method for determining the organic phosphorus content of five Alberta soils. The extractant was 2 N sulphuric acid and ignition was carried out at 600°C. for an hour. He extracted both the fresh and ignited soil repeatedly with the acid and found that four extractions were needed to remove the soluble phosphorus. The soluble phosphorus in the extracts after the first one was found to be similar in amount for fresh and ignited soils and thus a single extraction was thought to be sufficient to determine the organic phosphorus by difference. Odynisky's method has been used here in the determination of organic phosphorus of the same eight soils as were used in the alkali method.

Method of determination

Two one-gram samples of 20 mesh soil were weighed. One of the samples was ignited at 600°C. for one hour in an electric muffle. The ignited and unignited samples were then extracted with 200 c.c. of 2 N sulphuric acid by shaking for one hour and P_2O_5 in the filtered extracts was determined colorimetrically. Organic P_2O_5 was obtained by difference. As the extracts were too acidic for a direct colorimetric determination of phosphorus, they were adjusted to *p*H 3 with ammonia. The ammonium salts formed on neutralization interfered with the development and stability of the molybdenum blue colour. To obviate the error thus introduced an equal volume of 2 N sulphuric acid was added to the standard which was then neutralized with ammonia as before. This was not necessary when the extract was highly phosphatic and when only a small aliquot was required for the determination.

The results are shown in Table I and for comparison the organic phosphorus determined by the alkali method [Ghani, 1943, 1] is also presented in the same table.

TABLE I

Organic phosphorus by ignition method

(Mg. P_2O_5 per 100 gm. of soil)

Soil	Acid-soluble P_2O_5 in fresh soil	Acid-soluble P_2O_5 in ignited soil	Organic P_2O_5 by difference	Organic P_2O_5 by alkali method
A 1441, Rothamsted	51	118	67	68
A 2242, Woburn .	98	144	46	42
A 3457, Saxmundham	42	80	38	37
A 2328, Broadbalk .	133	176	43	45
A 3328, Carbello .	80	230	150	140
A 3769, Flatterton .	118	365	247	244
A 3246-7, Peterboro	307	444	137	108
A 3560-1, Littleport	96	221	125	84

It will be seen from Table I that in six out of the eight soils, the two methods give fairly agreeable results. It is only with the two heavy black fen soils from Peterborough and Littleport that the results do not agree and the discrepancy is more marked in the latter soil. These two soils are highly acid, their carbon content and clay content are also highest of the lot. The organic carbon content of the Peterborough and Littleport soils are 16·8 and 29·2 per cent respectively and they contain 34·0 and 45·5 per cent clay respectively. A fractionation study of the six soils reported previously also showed that these two soils contain high amounts of iron and aluminium phosphates. The higher values of organic phosphorus obtained by the ignition method were at first supposed to be due to some of the inorganic phosphates being included in it through its increased solubility by ignition as was pointed out by Fraps. To see whether ignition at lower temperatures would materially affect the acid-soluble phosphorus, ignition was carried out at 350°, 400° and 500°C. To ensure complete destruction of organic matter, the soils were kept at 400° and 350°C. for 2 hours and 3 hours respectively. The results are given in Table II which shows that temperature of ignition has practically no effect on the sulphuric acid soluble phosphorus and, therefore, if any change in the solubility of the inorganic phosphates has taken place it must have occurred at the lowest temperature.

TABLE II

Effect of temperature of ignition on phosphorus soluble in 2 N sulphuric acid

(Mg. P_2O_5 per 100 gm. of soil)

Temperature .	600°C.	500°C.	400°C.	350°C.
Time . .	1 hour	1 hour	2 hours	3 hours
A 3246-7 . .	444	444	448	452
A 3560-1 . .	221	218	220	220

EFFECT OF IGNITION ON THE ACID-SOLUBILITY OF PHOSPHORUS COMPOUNDS

Doughty [1935] determined the effect of heat on the solubility of phosphorus compounds of iron and aluminium in 0·002 N sulphuric acid. He found that ignition of ferric phosphate at 800°C. decreased the soluble phosphorus from 0·138 mg. to 0·072 mg. (at 600°C., 0·116 mg.) and in the case of aluminium phosphate ignition at 800°C. decreased the soluble phosphorous from 0·90 mg. to 0·012 mg. (at 600°C., 0·03 mg.). A sample of apatite gave no increase of soluble phosphorus when heated to 800°C., natural hydrated iron oxides containing some basic ferric phosphate showed a large increase in soluble phosphorus when heated under the same condition.

As the acid used in this work is much stronger, the effect of ignition on the solubility of normal and basic iron phosphates in 2 N sulphuric acid was determined. Where the whole of the phosphorus did not dissolve in one extraction, extraction of the residue was repeated with the acid. The ignition of the substances was carried out at 600°C. for one hour. The data are given in Table III.

TABLE III

Phosphorus dissolved by 2 N H_2SO_4 from fresh and ignited iron phosphate

(Per cent of P_2O_5 used)

Material		Fresh	Ignited
Normal iron phosphate	1st extract .	100	50·0
	2nd extract .	..	34·4
	3rd extract .	..	3·2
	Total .	100	87·6
Basic iron phosphate	1st extract .	100	40·4
	2nd extract .	..	57·0
	3rd extract .	..	2·0
	Total .	100	99·4

It will be seen from Table III that both normal and basic iron phosphates are completely soluble in 2N sulphuric acid. Ignition considerably decreased the solubility of both the substances. Basic iron phosphate, when ignited, required three extractions to dissolve the whole of its phosphorus, whereas in the normal iron phosphate, only about 88 per cent of its phosphorus was dissolved by three extractions. With basic iron phosphate, the

second extraction dissolved more phosphorus than the first one which suggests that the decreased solubility caused by ignition may be due to physical changes such as fusion of the particles into hard lumps. It will also appear, that simple phosphorus compounds behave differently on ignition from the natural phosphatic minerals; in the former case solubility is decreased whereas in the latter solubility is increased [Fraps, 1911].

The inorganic phosphates of the soil are present not only in the form of minerals but also as various colloidal complexes containing simple precipitated phosphorus compounds. It is quite likely that the increased solubility of the phosphorus in minerals such as wavellite, variscite and triplite tested by Fraps, is due to an increase in the surface exposed owing to disintegration by heat. On the other hand, with precipitated phosphates fusion will reduce the surface. In a complex material like soil which contains many kinds of mineral particles in different stages of disintegration and varying quantities of colloidal materials, it is scarcely possible to decide which process will predominate.

Use of acetic acid as a solvent

One way of getting rid of the contention that organic phosphorus determined by the ignition method includes some inorganic phosphorus is to choose an acid for extraction which would have no solvent action on the minerals. It is known that dilute acetic acid extracts from soil only the easily soluble phosphates and that it has no appreciable solvent action upon such minerals as wavellite and dufrenite, and other naturally occurring phosphates of iron and aluminium. Thus, if dilute acetic acid is substituted for 2 N sulphuric acid in the ignition method, the possibility of some inorganic phosphorus being included in the organic phosphorus thus determined may be eliminated. But in the absence of sufficient base in the soil, some of the phosphorus liberated by the destruction of organic matter may combine with the free iron and aluminium, forming iron and aluminium phosphates which are insoluble in acetic acid. To reduce these secondary reactions to a minimum, it was proposed to ignite the soil with chemically pure magnesium oxide and then to extract the ignited sample with N/2 acetic acid. The phosphorus liberated from organic compounds will combine with the base to form magnesium phosphate which is soluble in acetic acid. Again, inorganic soil colloids fix phosphorus from acetic acid solution, and the fixing power of a soil is considerably changed by ignition. Phosphorus will be fixed from acetic acid extract of both fresh and ignited samples during the period of extraction, but in different amounts. If the fixing power of the soil is increased by ignition, the organic phosphorus obtained by difference in the above method will be less than what it should be. To reduce this type of fixation, 8-hydroxy quinoline was added to the acetic acid. It has been shown by Ghani [1943, 2] that 8-hydroxy quinoline in acetic acid solution combines with the free sesquioxides present in the soil making them inactive. From these considerations the ignition method was modified as below.

A 5 per cent solution of 8-hydroxy quinoline in 2 N acetic acid was prepared. 5 c. c. of this solution and 195 c. c. of N/2 acetic acid was used for extracting one gram of soil. Two one-gram samples of the soil were mixed with 0·5 gm. of MgO and one was ignited at 600°C. for one hour in a muffle furnace. The ignited and the fresh samples were then extracted with 200 c. c. of the above 8-hydroxy quinoline-acetic acid solution by shaking for one hour in a rotatory shaker. The phosphorus in the extracts was determined colorimetrically and the organic phosphorus was obtained by difference. The results obtained with the fen soils are shown in Table IV. For comparison the results of acetic acid extraction without 8-hydroxy quinoline are also shown in the same table. The results show that the use of 8-hydroxy quinoline has increased the value of organic phosphorus in soil No. 3560-1 quite appreciably while in the other soil practically no change has been produced.

Table IV

Organic phosphorus by the modified ignition method

(Mg. P_2O_5 per 100 gm. of soil)

Soil	Extracted with acetic acid			Extracted with acetic acid and 8-hydroxy quinoline		
	Fresh	Ignited	Org. P_2O_5	Fresh	Ignited	Org. P_2O_5
A. 3246-7	28·0	160·0	132·0	32·0	163·2	131·2
A. 3560-1	24·8	102·4	77·6	34·4	140·8	106·4

The results obtained by the different methods are summarized and compared in Table V.

TABLE V

Organic phosphorus of fen soils determined by various methods

(Mg. P_2O_5 per 100 gm. of soil)

Soil	Ignition 2 N H_2SO_4	Ignition with MgO, N/2 acetic acid	Ignition with MgO, 8 (OH) quinoline-acetic acid	Alkali
A 3246-7 . .	137	132	131	108
A 3560-1 . .	125	78	106	84

It will be seen from Table V that in soil No. 3246-7 the organic phosphorus determined by the 8-hydroxy quinoline acetic acid method is nearly the same as that obtained by the sulphuric acid method, which indicates that the error due to increased solubility of mineral phosphates by ignition in the latter method is not serious in this soil. In the second soil, however, the values are quite appreciably reduced by the Modified method. The above modifications were introduced to explain the discrepancy of the results for the organic phosphorus as determined by the ignition and alkali method in the two fen soils. Comparing the modified ignition figures with the alkali figures it is found that the alkali figures are still lower. It was therefore thought that the low values of organic phosphorus by the alkali method might be due, at least in the fen soils, to inefficient acid pretreatment and incomplete extraction by the alkali. Schollenberger [1918] in comparing the efficiency of different acids as pretreating agents, found that in a highly organic soil acetic acid pretreatment was unsatisfactory for the determination of organic phosphorus by ammonia extraction. Lately, Wrenshall and Dyer [1941] showed that calcium phytate of soil is relatively insoluble in alkaline solution. If phytin or similar other organic phosphorus complexes of calcium happen to be present in large quantities in a soil, they will persist during alkali extraction unless the complexes are broken down and the calcium removed by a pretreatment. N/2 acetic acid used here may prove ineffective for this purpose and a stronger acid pretreatment may be necessary. Dyer and Wrenshall [1941] found that leaching with N HCl gave almost complete extraction of organic phosphorus by the subsequent alkali extraction. From this point of view the organic phosphorus was redetermined in the repeated alkali extracts after the soils were thoroughly extracted with normal hydrochloric acid instead of N/2 acetic acid. Determinations were made on all the eight soils.

ORGANIC PHOSPHORUS DETERMINED BY ALKALI METHOD AFTER PRETREATMENT WITH N HCl

One gram of soil was extracted with N hydrochloric acid each time with 100 c.c. of the solvent by shaking for two hours. The acid extracted soil was then repeatedly extracted with N/4 sodium hydroxide as usual and organic phosphorus determined in the extracts in the usual way. Unlike acetic acid, hydrochloric acid dissolved some organic phosphorus from the soil and hence the organic phosphorus in the acid extracts was also determined. As the acid extracts were a little coloured (especially in the organic soils) bromine oxidation had to be adopted for determining its organic phosphorus. It was also found necessary to remove the iron dissolved by the acid, in order that no interference might be caused in the colorimetric determination. The results are presented in Table VI. In the table the acid soluble organic phosphorus represent the organic phosphorus dissolved by two extractions with N hydrochloric acid and the alkali soluble part is the sum of the organic phosphorus of four successive extracts with N/4 sodium hydroxide. The total organic phosphorus obtained by this method has been compared in the same table with the corresponding figures using acetic acid as a pretreating agent, together with the ignition method figures.

TABTE VI

Organic phosphorus determined by alkali method after pretreatment with N HCl

(Mg. P_2O_5 per 100 gm. of soil)

Soil	A 1441	A 2242	A 3457	A 2328	A 3328	A 3769	A 3246-7	A 3560-1
N HCl	1·9	0·0	2·9	2·0	20·0	46·0	0·0	8·5
N/4 NaOH	64·6	33·7	30·8	39·2	110·5	194·9	119·5	96·4
Total .	66·5	33·7	33·7	41·2	130·5	240·9	119·5	104·9
Acetic acid pretreatment . .	68	42	37	45	140	244	108	84
Ignition method (2 N H_2SO_4) .	67	46	38	43	150	246	137	125
Ignition method (Hydroxy quinoline acetic acid)	..	..	..	..	..	..	131	106

It will be seen from Table VI that replacement of acetic acid by hydrochloric acid in the pretreatment has increased the values of organic phosphorus in both the fen soils but not by as much as will bring them to the level of the ignition figures (H_2SO_4). In the remaining six soils, the nature of the pretreating agent had practically little effect upon the amount of organic phosphorus determined. With soil No. A 3560-1, the modified alkali method (HCl pretreated) gives results comparable with the modified ignition method (8-hydroxy quinoline acetic acid). The other soil too does not show widely divergent results. It would thus appear that the alkali method and the ignition method can be reliably used in a majority of soils. Only the highly organic soils appear to give somewhat lower values by the ordinary alkali method and somewhat higher ones by the ordinary ignition method. The ignition method gives better results when hydroxy quinoline acetic acid is used as an extractant instead of sulphuric acid. In such soils, the alkali method should also be modified by using a stronger acid for pretreatment.

It was next intended to see how far the hydrogen peroxide oxidation method can be used for the determination of the organic phosphorus of the soil.

Determination of organic phosphorus by hydrogen peroxide oxidation method

This method is similar in principle to the ignition method; the organic matter of the soil is oxidized with hydrogen peroxide instead of by ignition. Peterson [1912] found that nearly 90 per cent of the organic matter was removed by hydrogen peroxide and that the phosphorus extracted by 0·2 N nitric acid was greatly increased by such treatments. Hydrogen peroxide oxidation always gave a greater increase in acid-soluble phosphorus than did ignition. Dushechkin and Gorodinsky [1936] used hydrogen peroxide as an oxidizing agent in separating the organic form of phosphorus from the mineral one. The soil was previously treated with 0·05 N hydrochloric acid in order to remove the most easily soluble part of the mineral phosphoric acid. The acid treated soil was then digested with 30 per cent perhydrol till apparent disappearence of humus. The oxidized soil was then washed with 0·05 N hydrochloric acid till the reaction for phosphoric acid disappeared. The phosphoric acid determined in the leachate was supposed to be in organic form. Dickman and De Turk [1938] made a further study of the hydrogen peroxide method and determined the organic phosphorus content of ten soils by this method. Phosphorus was extracted from the soil with 0·2 N sulphuric acid, and the difference between the phosphorus contents of the extracts of the oxidized and unoxidized soil was taken as a measure of the organic phosphorus.

In the method adopted here, 0·05 N hydrochloric acid has been used as an extractant.

Method of determination

One gram of soil was weighed into a 100 c.c. Erlenmeyer flask and 10 c.c. of 90 to 100 volume hydrogen peroxide (phosphorus free) added to the soil. After the reaction ceased, the flask was heated on a sand bath with occasional shaking, care being taken to prevent loss by frothing by removing the flask from the sand bath occasionally. Hydrogen peroxide was then added by small portions (5 c.c.) every 10 min. until apparent disappearance of the organic matter. The contents of the flask were evaporated to a thick paste, washed with 0·05 N hydrochloric acid into a shaking bottle and diluted with the same acid up to the 250 c.c. mark. The bottle was shaken in a mechanical shaker for two hours, filtered and P_2O_5 determined colorimetrically on an aliquot of the filtrate. Before the colorimetric determinations were done traces of hydrogen peroxide in the extract were removed by evaporating the latter to dryness.

Another one gram sample of the soil was extracted with 250 c.c. of 0·05 N hydrochloric acid by shaking for two hours and P_2O_5 determined in the filtrate. Organic phosphorus was obtained by difference.

The results for the eight soils under study, obtained by the above method, are shown in Table VII and compared with those obtained by the ignition method.

Table VII

Organic phosphorus by hydrogen peroxide oxidation method

(Mg. P_2O_5 per 100 gm. of soil)

Soil	Acid-soluble P_2O_5 in fresh soil	Acid-soluble P_2O_5 in oxidized soil	Organic P_2O_5 by difference	Organic P_2O_5 by ignition method
A 1441 . . .	7	40	33	67
A 2242 . . .	15	42	27	46
A 3457 . . .	9	23	14	38
A 2328 . . .	31	75	44	43
A 3328 . . .	8	132	124	150
A 3769 . . .	30	305	275	246
A 3246-7 . .	61	370	309	137
A 3560-1 . .	52	212	160	125

It will be seen from Table VII that, except in soil No. A 2328, the two methods give discordant results for organic phosphorus. With the three

highly organic soils (A 3769, A 3246-7 and A 3560-1) the hydrogen peroxide method gives much higher values than the ignition method, while with the remaining four soils the converse is true.

Discussion of the hydrogen peroxide method

In adopting this method for the determination of organic phosphorus in soils, the following assumptions were made.

(1) Hydrogen peroxide decomposes the organic matter completely and releases all the organic phosphorus in an acid soluble form.

(2) Hydrogen peroxide oxidation has no effect on the solubility of the inorganic phosphates.

(3) None of the liberated phosphorus is fixed by the soil.

As regards the first assumption, there is no definite evidence that hydrogen peroxide destroys the organic matter completely. Peterson [1912] investigated this point and found that removal of 60 per cent of organic matter gave as much soluble phosphorus as the removal of 80 per cent. From this evidence he concluded that 'it seems reasonable to believe that removal of the entire amount of organic matter will not increase the solubility of the phosphoric acid above that already obtained.' But this may not be true for all types of soils. In the soils used here the lower organic phosphorus values (as compared with the ignition method) were obtained from the soils which are low in organic matter, while those high in organic matter gave higher values. So it does not seem that the discrepancy is due to incomplete oxidation of the organic matter.

As regards the second assumption, it is quite probable that some of the organic phosphates will be protected from the solvent by the organic matter before oxidation. Peterson [1912] studied the effect of different degrees of oxidation on the solubility of phosphorus, iron, aluminium, calcium and manganese. He found that the increase in the soluble iron and aluminium was roughly parallel to the increase in soluble phosphorus while the calcium and manganese remained constant in solubility. This led him to believe that iron and aluminium were not combined with the phosphorus in a single organic molecule but that some of the phosphates of iron and aluminium were merely protected from the solution before oxidation by the organic matter and were dissolved by the acid when the organic matter was decomposed. It seems that the higher values of organic phosphorus of the three soils are due to this reason. They are highly organic and contain a large amount of iron phosphates as determined by alkali extraction. Soil No. A. 3246-7 which shows the greatest discrepancy contains the highest amount of iron phosphate [Ghani 1943, 1].

As regards the third point, Dickman and De Turk [1938] found that some of the phosphorus liberated during the oxidation was fixed in a form which was insoluble in 0·002 N sulphuric acid. Even with 0·2 N sulphuric acid, reliable results could not be obtained by them and it was found that appreciable fixation of the liberated phosphorus took place, especially in soils low in organic matter and high in active iron and aluminium. They suggested a correction factor (based on the fixing power of the soil) to be applied to the organic phosphorus value as determined by hydrogen peroxide oxidation. They also suggested necessary modifications of the method to meet particular local conditions. It appears that the low values for organic phosphorus obtained in four soils are due to the fixation of the liberated phosphorus in a form not soluble in 0·05 N hydrochloric acid used in this work.

In determining organic phosphorus by hydrogen peroxide oxidation these sources of errors must be taken into account. To reduce these possibilities, the procedure was slightly modified. The oxidation was carried out on the same sample as was extracted with acid. The preliminary extractions were repeated to deplete the soil of the easily soluble phosphorus. This would remove, to a certain extent, the possibility of some inorganic phosphorus being included in the final extract, particularly in the three organic soils from which the first acid extraction dissolved quite a high amount of phosphorus. The oxidized soil was also repeatedly extracted to dissolve the fixed phosphorus as far as possible.

One gram of soil was repeatedly extracted with 0·05 N hydrochloric acid. Three extractions were needed to bring down the soluble phosphorus to a small quantity. The residual soil was washed off the bottle with water into a conical flask and the water evaporated off. The oxidation with hydrogen peroxide was carried out as before. The contents of the flask were then washed off into a shaking bottle with 0·05 N hydrochloric acid and extracted as before. The extraction was repeated thrice, and phosphorus determined in the extract.

The data obtained are presented in Table VIII. Comparing Table VII with Table VIII it will be seen that the modified procedure gave lower values for the three organic soils while in the remaining five there was a slight increase. However, on comparing with the organic phosphorus values by the ignition and alkali method (Table VI) it will be found that hydrogen peroxide oxidation gave much lower values in soils A. 1441, A. 2242, A. 3457 and A. 3328, higher values in soils A. 3246-7 and A. 3560-1 and comparable values in soils A. 2328 and A. 3769. From the conflicting results obtained here with different types of soil

it may reasonably be said that hydrogen peroxide oxidation method for the determination of the organic phosphorus in its present stage, cannot be universally applied to obtain reliable results and that the conditions for extraction and oxidation need thorough investigation as regards fixation and other factors discussed here.

TABLE VIII

Organic P_2O_5 by H_2O_2 oxidation (repeated extraction with 0·05 N HCl)

(Mg. P_2O_5 per 100 gm. of soil)

Soil	1st Extract	2nd Extract	3rd Extract	Total organic P_2O_5
A. 1441 . .	20	10	8	38
A. 2242 . .	16	9	7	32
A. 3457 . .	10	4	3	17
A. 2328 . .	30	10	7	47
A. 3328 . .	104	14	10	128
A. 3769 . .	198	27	17	242
A. 3246-7 . .	208	26	14	248
A. 3560-1 . .	131	7	3	141

SUMMARY

With a view to testing the validity of the fractionation method in determining the organic phosphorus of the soil, an independent method based on ignition has been used for the determination of the fraction.

The ignition method gave results for organic phosphorus which agreed fairly well with those by the alkali method except in the two fen soils. The causes of this discrepancy were examined and the methods modified accordingly.

The temperature of ignition when varied from 350° to 600°C. did not effect the results for organic phosphorus. Ignition considerably reduced the solubility of basic and normal iron phosphates in 2 N sulphuric acid.

To obviate the possibility of error due to change in acid-solubility of the inorganic phosphorus compounds and phosphatic minerals caused by ignition, N/2 acetic acid was substituted for 2 N sulphuric acid as an extractant. Also to prevent any possible fixation of phosphorus from the acetic acid extract, 8-hydroxy quinoline was introduced in the extracting acid and the ignition of the soil was carried out in presence of magnesium oxide. The ignition method thus modified gave lower values than before in both the soils, though they were still higher in comparison with the alkali method figures.

The alkali extraction method was also modified with respect to the pretreating acid. Pretreatment of the soil with N HCl instead of with N/2 acetic acid, increased the values for organic phosphorus in the two fen soils; in the remaining six soils nature of the pretreating agent had a slight effect on the amount of organic phosphorus determined. The modified alkali method gave results comparable with the modified ignition method in the two fen soils.

From the work reported, it appears that both the methods can be used reliably in the majority of soils. Only in the case of a special type of highly organic fen soils, the methods need modification.

The validity of using hydrogen peroxide oxidation method for the determination of organic phosphorus was next examined. It was found that from various points of view this method cannot be universally applied to obtain reliable results.

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THE MANURIAL VALUE OF DIFFERENT MINERAL PHOSPHATES IN CALCAREOUS SOILS

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(With one text-figure)

Soils in the Indo-Gangetic alluvium around Pusa in Bihar contain 30 to 40 per cent of calcium carbonate. This is likely to enter into combination with many of the constituents of fertilizers used in ordinary farm practice, rendering them insoluble and consequently perhaps unavailable to plants. This untoward feature has baffled many attempts in the past to evolve a successful method of manuring these calcareous soils, especially with regard to their supply of phosphorus. Superphosphate is the phosphatic fertilizer commonly recommended for such soils.

Experience has shown that the beneficial action of superphosphate on calcareous soils is not always appreciable, although good crop yields are usually obtained when applied in conjunction with organic manures as reported by Hutchinson [1923]. Commercial super contains 50 to 60 per cent of gypsum. Studies made by the author [Das, 1933] to determine the effect of gypsum on the cropping value of calcareous soils, showed that it exerted no beneficial influence, but often depressed the yield. The presence of gypsum therefore may account in some measure for the uncertain and disappointing results often obtained with commercial super in calcareous soils.

Harrison and Das [1921] have shown that the action of super on calcareous soils is very localized; the major portion of its P_2O_5 is retained in the top layers with the formation of dicalcium phosphate. The latter then becomes slowly converted into insoluble tricalcium phosphate on reacting with excess of calcium carbonate already present. Consequently, the application of super to these soils has a very restricted effect and probably much less efficiency than equivalent amounts applied to non-calcareous soils.

On the other hand, it was shown by the authors [Harrison and Das, 1921] that, by using dressings of soluble phosphates like those of sodium which have little or no action with calcium carbonate, in place of super, on calcareous as well as non-calcareous soils, a much more uniform distribution of P_2O_5 throughout the soil can be attained. Provided that these phosphates are suitable as plant food, this wider distribution can only result in a wider and deeper root action and better cropping in these soils. It was therefore considered desirable to test how far these soluble sodium and similar phosphates could be successfully utilized in calcareous soils as phosphatic fertilizers.

In order to do this, some soluble phosphates such as meta- and pyro-phosphates of sodium were prepared as described below; these contained 67·20 and 51·98 per cent of P_2O_5 respectively, the theoretical amounts being 69·61 and 53·43 per cent.

Sodium hexametaphosphate $(NaPO_3)_6$. To a solution of common sodium phosphate Na_2HPO_4, $12HO_2$ add the requisite quantity of phosphoric acid H_3PO_4 to form sodium dihydrogen phosphate NaH_2PO_4. The latter is heated to complete fusion. The melted mass must be quickly cooled to avoid the formation of trimetaphosphate $(NaPO_3)_3$. The product is a vitreous mass, soluble in water and slightly acid in reaction.

Sodium pyrophosphate $Na_4P_2O_7$. It is obtained in the anhydrous state as a colourless glassy mass by igniting common sodium phosphate and quickly cooling the ignited mass. It dissolves in water and is alkaline in reaction.

Besides, sodium phosphate Na_2HPO_4, $12H_2O$, dicalcium phosphate $CaHPO_4$, $2H_3O$, and tricalcium phosphate $Ca_3(PO_4)_2$ were used in pot experiments. Parallel experiments with super were also carried out for comparison. In pot experiments monocalcium phosphate $CaH_4(PO_4)_2$ H_2O which is devoid of gypsum, but contains the chief phsophatic constituent of commercial super, was used in place of super.

A sample of Pusa soil containing 33 per cent of calcium carbonate was collected from a fallow plot for pot experiments in 1919. Four pots of almost similar dimensions, e.g. 12 in. diameter by 22·5 to 24·0 in. high formed a group and received similar treatment. The pots contained 49 kilos of air-dry soil in each. Nitrogen was applied at the rate of 80 lb. per acre in the form of ammonium sulphate in order to prevent lack of nitrogen. Phosphates were added to the top three inches of soil as used in farm practice here at 80 lb. of P_2O_5 per acre, and the pots watered from the top to get the effect of different degrees of diffusion. *Ragi* (*Eleusine coracana*) was chosen as the *kharif* (summer) crop.

Although the weights of both grain and straw per pot were recorded saparately, only the yield

of grain is given in Table I. While examining the data, Fisher's [1932] analysis of variance was adopted.

TABLE I

The effect of different phosphates on the cropping value of a calcareous Pusa soil in pot experiments with ragi

Treatment	Replications				Mean yield in gm.	Per centage increase over control
	1	2	3	4		
Control . . .	18·2	16·3	16·7	17·6	17·2	...
Dicalcium phosphate	38·0	31·9	35·6	42·6	37·0	115·1
Monocalcium phosphate (super)	64·3	58·3	57·4	56·0	59·0	243·0
Sodium phosphate .	53·5	57·0	64·0	59·2	58·4	239·5
Sodium metaphosphate . . .	70·0	61·8	64·1	71·2	66·8	288·4
Sodium pyrophosphate . . .	70·4	68·6	75·4	71·8	71·6	316·3

Standard error of difference for comparison of mean yields by Fisher's [1932] analysis of variance = 2·62
Critical difference for P = 1 per cent = *7·54*
Ditto P = 4 per cent = *5·51*

The *ragi* crop, on account of its high salt tolerance, produced remarkably high yields in pots to which fertilizer salts were added. This is evident on examining the high percentage increase of yield over the control in the last column of Table I. It will be further noticed that the differences in mean yields of grain between control and every other treatment are significant at one per cent level. Such significant relationships have been obtained between other treatments too with the exception of those between monocalcium phosphate and sodium phosphate as well as between sodium meta- and pyro-phosphates. Although all the phosphates have given significantly better yields than the control, sodium meta- and pyro-phosphates give higher crop returns than the other phosphates.

Monocalcium phosphate which was used in place of commercial superphosphate in order to eliminate the deleterious influence of gypsum contained in super has produced results comparable with phosphates of sodium. When the efficiency of other phosphates over monocalcium phosphate is compared, only meta- and pyro-phosphates give significantly better yields than monocalcium phosphate. The effect of sodium phosphate is uncertain, and that of dicalcium phosphate is negative.

In the winter of 1919 these pot experiments were repeated, but without nitrogen. Oats were chosen as the *rabi* (winter) crop. The yield of grain of individual pots is set forth in Table II.

TABLE II

The effect of different phosphates on the cropping value of a calcareous Pusa soil in pot experiments with oats

Treatment	Replications				Mean yield in gm.	Percentage increase over control
	1	2	3	4		
Control . . .	27·0	26·8	29·2	26·0	27·3	...
Monocalcium phosphate (super)	36·0	34·2	33·2	35·1	34·6	26·8
Dicalcium phosphate	34·7	29·8	36·1	35·5	34·0	24·5
Tricalcium phosphate	33·0	34·6	34·0	33·6	33·8	23·8
Sodium phosphate .	30·4	32·6	33·4	35·3	32·9	20·5
Sodium metaphosphate . . .	37·2	34·0	39·2	39·5	37·5	37·4
Sodium pyrophosphate . . .	37·7	34·5	39·9	36·8	37·2	36·3

Standard error of difference for comparison of mean yields by Fisher [1932] analysis of variance = 1·403
Critical difference for P = 1 per cent = *3·97*
Ditto P = 5 per cent = *29·1*
Ditto P = 10 per cent = *24·2*

The differences in mean yields of oats between control and every other treatment are similar to those in the preceding pot experiments with *ragi*. Although all the phosphates have given significantly better yields than the control, sodium meta- and pyro-phosphates have again yielded higher crop returns than the remaining phosphates, thus confirming the conclusions emerging from the previous set of experiments with *ragi*.

Experiments on a field scale were next instituted during the monsoon of 1921 with *ragi*. Eighteen plots, each 2½ ft. wide and 42 ft. long, were laid out in three equal blocks having a margin of 2 ft. between them. Containing one plot of each of the six treatments distributed in a randomized manner, every block had thus six plots each with a border of one foot all around. During harvest, however, the crop of this border area was rejected in order to eliminate the border effect. Non-experimental margins were not however provided for each plot. *Ragi* seeds were sown in breadthwise line 6 in. apart and different phosphatic fertilizers were applied at the rate of 40 lb. of P_2O_5 per acre. The weights of grain are given according to blocks in Table III.

Sodium meta- and pyro-phosphates again produce markedly better yields than the other phosphates, and the results of these field experiments confirm the conclusions drawn from pot experiments. Moreover, from these cropping experiments the adverse effect of sodium ion resulting from the application of various sodium phosphates is not quite apparent on calcareous

TABLE III

The effect of different phosphates on calcareous Pusa soil in field experiments with ragi

Treatment	Replications 1	2	3	Mean yield in gm.	Percentage variation over control
Control	617	658	599	624·7	...
Superphosphate	549	626	612	595·7	— 4·7
Tricalcium phosphate	699	599	658	652·0	+ 4·4
Sodium phosphate	735	671	717	707·7	+13·3
Sodium metaphosphate	798	785	826	803·0	+28·5
Sodium pyrophosphate	830	735	762	775·7	+24·2

Standard error of difference for comparison of mean yields by Fisher's [1932] analysis of variance = 32·7

Critical difference for P = 1 per cent = *103·6*
Ditto P = 5 per cent = *72·9*
Ditto P = 10 per cent = *59·3*

soils. This is perhaps due to their high content of calcium carbonate, which has no action on meta- and pyro-phosphates of sodium and reacts only slowly with sodium phosphate, as will be shown in the sequel.

On the other hand, superphosphate has given a lower yield than even the control, and tricalcium phosphate also does not show a significant increase. Thus, both the phosphates indicate their erratic effect on calcareous soils. In pot experiments where monocalcium phosphate was used which contains the chief phosphatic constituent of commercial superphosphate but not gypsum, its crop-depressing constituent, a significantly better crop yield than the control was secured. In these field tests, however, commercial superphosphate was used instead. This perhaps explains the disappointing result, which is the common experience in local agricultural practice, when super is used alone.

The pot and the field experiments have thus satisfactorily established the beneficial effect of the phosphates of sodium in calcareous soils. These results are very remarkable from the point of view of manuring calcareous soils with phosphatic fertilizers in order to supply their phosphatic needs.

Potassium and ammonium phosphates may also behave equally well in these soils. Commercial ammonium phosphates in their different grades of low nitrogen and high phosphate contents will, besides phosphates, supply nitrogen as well to which these calcareous soils readily respond. Work is in progress in this laboratory to manufacture similar phosphatic fertilizers from indigenous sources suitable for calcareous and other soils in India.

Having thus shown the beneficial action of several phosphates of sodium by pot and field experiments, it was next considered worthwhile to study the action of calcium carbonate normally present in calcareous soils on these phosphates. For this purpose, two series of 8 experiments in each group were carried out. One gram of pure precipitated calcium carbonate was shaken separately with 100 c.c. of sodium meta- and pyro-phosphate solutions containing 106·7 and 97·9 mg. of P_2O_5 respectively for 1, 3, 6, 16, 20, 24, 48, and 96 hours in end-over-end rotations in a mechanical shaker in a room which was not subjected to sudden changes of temperature. At the end of varying periods, the filtrates were examined for P_2O_5 content.

The concentration of P_2O_5 in the solutions remained constant both before and after shaking with calcium carbonate, showing the complete absence of interaction. Under the experimental conditions the possibility of the formation of compounds like calcium meta- and pyro-phosphate at ordinary laboratory temperature is rather remote, as they usually require a very high temperature for their preparation. Consequently, the absence of any reaction renders a more uniform distribution of these phosphates and ensures their better manurial value in calcareous soils.

The conclusion is therefore forced that the higher soluble phosphates of the type containing either PO_3 or P_2O_7 ions which have no reaction with calcium carbonate give the best results in calcareous soils by effectively supplying the phosphatic nutrition of plants, notwithstanding the presence of a large amount of calcium carbonate in them. This discovery opens up an altogether new line of attacking the important problem of manuring calcareous soils with phosphatic fertilizers of the meta- and pyro-type. Hitherto phosphatic manuring of soils all over the world has been mainly restricted to phosphatic fertilizers of the type having PO_4 ions in them.

Next the action of sodium phosphate, Na_2HPO_4, $12H_2O$ on calcium carbonate was studied. At ordinary laboratory temperature one gram of pure, precipitated calcium carbonate was shaken with 100 c.c. of a sodium phosphate solution containing 108·5 mg. of P_2O_5 for varying periods of time, at the end of which P_2O_5 concentration of the filtrates was determined. The difference between the P_2O_5 originally taken and that found in the filtrate after shaking gives the amount of P_2O_5 precipitated by calcium carbonate. The results are given in Table IV.

A slow, but progressive reaction takes place which proceeds in a well defined manner. It would appear that the reaction results in the formation of dicalcium phosphate in the first

TABLE IV

The reaction between sodium phosphate solution and calcium carbonate when shaken together for varying periods of time

Number of experiments	Time of shaking in hours (t)	Gm. P_2O_5 taken in solution	Gm. P_2O_5 in solution after shaking	Gm. P_2O_5 retained (y)	Per cent P_2O_5 retained by $CaCO_3$
1	6	0·1085	0·0935	0·0150	13·8
2	16	0·1085	0·0781	0·0304	28·0
3	24	0·1085	0·0719	0·0366	33·7
4	48	0·1085	0·0631	0·0454	40·9
5	96	0·1085	0·0518	0·0567	52·3
6	192	0·1085	0·0430	0·0655	60·4
7	384	0·1085	0·0362	0·0723	66·6
8	768	0·1085	0·0251	0·0834	76·9
Blank	768	0·1085	0·1085	*Nil*	No $CaCO_3$ added

instance, which, on reacting with excess of calcium carbonate already present, is slowly converted into tricalcium phosphate according to the following equations:

$$Na_2HPO_4 + CaCO_3 = CaHPO_2 + Na_2CO_3 \quad (1)$$

Dicalcium phosphate

$$2CaHPO_4 + CaCO_3 = Ca_3(PO_4)_4 + CO_2 + H_2O \quad (2)$$

Tricalcium phosphate

The solubility of dicalcium phosphate in water at the ordinary temperature is only 34 mg. of P_2O_5 per 100 c.c. and that of tricalcium phosphate is still less. The amount of P_2O_5 actually found in the solution after continuous shaking for 768 hours or 32 days is 25 mg. only per 100 c.c. which indicates that the reaction takes place in the manner shown above.

It is further noticed that the concentration of P_2O_5 in solution decreases with the period of time. The relationship between the amount of P_2O_5 retained (y) and the time (t) is a simple semi-logarithmic one as shown by plotting the values of y against the log of time (Fig. 1).

This relationship may be expressed by the general formula,

$$\log t = ay - k,$$

where y is the amount of P_2O_5 precipitated by $CaCO_3$ in time t, and a and k are constants. Employing the values of $a = 31 \cdot 22$, and $k = -0 \cdot 264$, the calculated and the experimental vlaues of y are given in Table V which show close agreement among themselves.

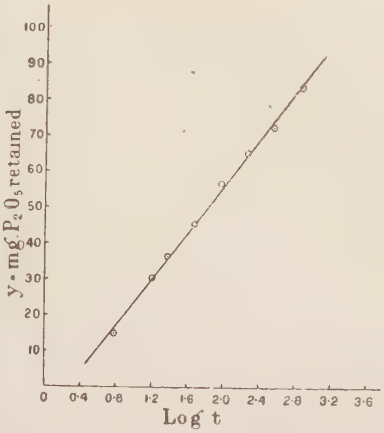

FIG. 1. Graph obtained by plotting the amounts of P_2O_5 retained by $CaCo_3$ against log of time.

TABLE V

A comparison of the calculated and the experimental values for the retention of P_2O_5 *by* $CaCO_3$

Number of experiments	t	y calculated	y found	Error
1	6	0·0165	0·0150	−0·0015
2	16	0·0300	0·0304	+0·0004
3	24	0·0357	0·0366	+0·0009
4	48	0·0454	0·0454	0·0000
5	96	0·0550	0·0567	+0·0017
6	192	0·0646	0·0655	+0·0009
7	384	0·0742	0·0723	−0·0019
8	768	0·0840	0·0834	−0·0006

From the relationship $\log t = ay - k$, it follows that under the experimental conditions $y = 0 \cdot 032 \log t - 0 \cdot 0085$, and hence the velocity of the reaction is $dy/dt = 0 \cdot 032t^{-1}$, and the acceleration is $d^2y/dt^2 = 0 \cdot 032t^{-2}$. The velocity of the reaction varies inversely as the time, and the acceleration is negative and is in inverse proportion to the square of time. The reaction is thus strongly retarded and can only reach completion at the end of a considerable period of time, which, when calculated from the experimental data, is found to be over six months. This conclusion is arrived at when the reacting bodies are in continuous and intimate contact but the retention of such soluble phosphates must proceed at a much slower rate under the conditions which obtain in the soil. This is due to the fact that carbonates in the soil are protected by soil colloids or locked up within other minerals. Consequently, they will not be so freely exposed to the action of sodium phosphate solution as the finely precipitated, pure calcium carbonate used in the experiments.

Therefore, soluble phosphates of the above type reacting slowly, or none at all with calcium carbonate, as in the case of sodium meta- and pyro-phosphates, will have enough time during the growing season to get themselves easily distributed more uniformly into deeper layers, resulting in a wider and deeper root action and better cropping in calcareous soils.

Summary and conclusions

1. The effect of superphosphate on crop yields in calcareous soils is uncertain and erratic, although better results are usually obtained with it when applied in conjunction with heavy organic manures. This is due, among other factors, to the retention of water-soluble P_2O_5 in the upper layers of soils as insoluble calcium phosphates above the root-range of many crops and also to the deleterious influence of gypsum present in commercial super.

2. On the other hand, pyro- and meta-phosphates of sodium having no reaction at all with calcium carbonate, and di-sodium hydrogen phosphate which reacts only slowly with soil carbonates render a more even distribution of their P_2O_5 throughout the deeper layers of calcareous soils, resulting in a wider and deeper root action and better cropping. This has been satisfactorily demonstrated in calcareous soils both by pot and field experiments.

3. Higher soluble phosphates of the type of sodium meta-and pyro-phosphates containing respectively PO_3 and P_2O_7 ions which do not react at all with calcium carbonate, give the best crop results in calcareous soils. This suggests a new method of manuring calcareous soil types with suitable phosphatic fertilizers.

4. A slow, but progressive action takes place between sodium phosphate solution and the calcium carbonate normally present in calcareous soils. The relationship between the amount of P_2O_5 retained by soil carbonates and the time of reaction is a simple semi-logarithmic one, and can be expressed by the general equation,

$$\log t = ay - k,$$

where y is the amount of P_2O_5 retained in time t, and a and k are constants.

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ON THE BACTERIOPHAGE OF ROOT NODULE ORGANISMS

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Desai [1932] reported investigations on the nature of bacteriophages of root nodule organisms in which he evolved a method for the isolation and enhancement of the virulence of bacteriophages of certain leguminous plants. Following the method evolved by him the work on the bacteriophages of root nodule organisms proceeded on the following lines : (*i*) whether bacteriophage occurs in all legumes, (*ii*) if it occurs whether it is the same or different in different types of nodules, and if different, whether they form any basis for the classification of nodule bacteria, (*iii*) the relationship of bacteriophage to the formation or otherwise of nodules, and (*iv*) any practical utility of bacteriophage in nature.

A part of the work reported in this paper was carried out in 1932 and 1933 at Pusa in Bihar and the work was stopped in 1934 due to the great earthquake. The work was resumed at Delhi in 1940 when some of the conclusions arrived at Pusa had to be modified in the light of newer knowledge gained by working on legumes grown on different types of soil.

Work done at Pusa. Various leguminous plants were tested for the presence of bacteriolytic principle in their nodules. While some of them contained specific bacteriophage others did not show presence of any in them. The results are given in Table I. It will be seen from this table that berseem (*Trifolium alexandrianum*), Sweet pea (*Lathyrus odoratus*), Lucerne (*Medicago sativa*), Karao (*Pisum arvense*), Gram (*Cicer arietum*), Dolichos (*Dolichos Lablab*), contained bacteriophage in their root nodules, but cowpea (*Vigna catjang*), Sannhemp (*Crotalaria Juncia*), *Mung* (*Phaseolus radiatus*), Pea (*Pisum sativum*), Fennugreek (*Trigonella Foenum graecum*), Math (*Phaseolus aconitifolius*), Indigo (*Indigofera Tinctoria*), *Arhar* (*Cajanus indicus*) did not show presence of bacteriophage in their nodules. Attempts were made many times to isolate phage from these plants, but no success could be attained. It was then presumed that certain legumes have got phage in their nodules while others have not. These tentative conclusions have had to be modified when newer knowledge was gained by the work at Delhi. Karnal and Lyallpur.

Time of appearance of bacteriophage in the legumes. It will be noticed from Table I that the young plants did not indicate appearance of the bacteriophage in any types of plants experimented with. These results, so far as they go, are at variance with those of Laird [1932] who observed that bacteriophage might be isolated readily from young plants, and with difficulty or not at all from old ones.

Specificity of bacteriophages. Having obtained six different cultures of bacteriophages their specificity in the production of lysis on the solid medium of cultures of different species of nodule organisms individually and separately was tested. The results are given in Table II. It will be seen that berseem bacteriophage and sweet pea bacteriophage touched cultures of berseem and sweet pea organisms in direct and cross inoculations, gram bacteriophage touched both gram and lucerne organisms while lucerne phage did not touch the gram organisms, the other bacteriophages touched only their own organisms but not others. It seems some of the bacteriophages are specific for their own organisms.

TABLE I

Bacteriophage in legumes

Legume	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks
1. Berseem	− − −	+ + +	+ + +	+ + +	+ + +
2. Sweet pea	− − −	+ + +	+ + +	+ + +	+ + +
3. Lucerne	− − −	− − −	− − +	+ + +	+ + +
4. Karao	− − −	− − −	− − +	+ + +	+ + +
5. Gram	− − −	− − +	+ + +	+ + +	+ + +
6. Dolichos	− − −	− − +	+ + +	+ + +	+ + +
7. Cowpea	− − −	− − −	− − −	− − −	− − −
8. Sannhemp	− − −	− − −	− − −	− − −	− − −
9. *Mung*	− − −	− − −	− − −	− − −	− − −
10. Pea	− − −	− − −	− − −	− − −	− − −
11. Fennugreek	− − −	− − −	− − −	− − −	− − −
12. Meth	− − −	− − −	− − −	− − −	− − −
13. Indigo	− − −	− − −	− − −	− − −	− − −
14. *Arhar*	− − −	− − −	− − −	− − −	− − −

TABLE II

Organism	Bacteriophage					
	Berseem	Sweet pea	Lucerne	Karao	Gram	Dolichos
Berseem	0	0	..	..	..	..
Sweet pea	0	0	..	..	..	..
Lucerne	..	..	0	..	0	..
Karao	..	..	..	0	..	..
Gram	..	..	..	..	0	..
Dolichos	..	..	..	..	..	0

Work done at Delhi. On the transfer of the Institute from Pusa to Delhi in 1936 it was necessary to freshly isolate bacteriophage from the root nodules of berseem as the cultures of berseem bacteriophage were contaminated in transit. The Agricultural Farm of the Institute started growing berseem in Delhi area, for the first time in 1936-37. Previously no such fodder crop was grown in this area. Samples of berseem nodules were collected in 1936-37 in January and in March. The samples were analysed for the presence of bacteriophage. But no bacteriophage could be isolated. This trial was repeated in 1937-38 and 1938-39 without any success. While at Pusa berseem bacteriophage was easily isolated from the nodules of berseem plants growing in the berseem fields. These plots were holding berseem stands for a number of years. The failure to isolate any bacteriolytic principle from the berseem plants growing at the Delhi Farm led to the supposition that bacteriophage is produced in certain soil but not in others. With this end in view it was decided to study the berseem plants growing in Lyallpur and Karnal soils. In March 1941 six samples of berseem nodules were

collected from Lyallpur farm from plots growing berseem for two, six and twelve years; and four samples from Karnal farm from plots growing berseem for one, two, three and five years. These samples were stored in sterile test tubes and brought to Delhi for analysis. The results of analysis of these samples for the presence of bacteriophage are given in Table III.

TABLE III

Presence of bacteriophage in the nodules of berseem plants growing at Lyallpur and Karnal farms

	Place	Phage after 24 hr.	Phage after 48 hr.	Phage after 72 hr.
1. Berseem growing in the plots for 2 years	Lyallpur	+ − −	+ − −	+ − −
2. Berseem growing in the plots for 2 years	Lyallpur	− − −	+ − −	− − −
3. Berseem growing in the plots for 6 years	Lyallpur	+ + −	+ + +	+ + +
4. Berseem growing in the plots for 6 years	Lyallpur	+ − −	+ + −	+ + +
5. Berseem growing in the plots for 12 years in rotation	Lyallpur	+ − −	+ + −	+ + −
6. Berseem growing in the plots for 12 years in rotation	Lyallpur	+ − −	+ + −	+ + −
7. Berseem growing in the plots for 1 year	Karnal	− − −	− − −	− − −
8. Berseem growing in the plots for 2 years	Karnal	− − −	− + −	− + −
9. Berseem growing in the plots for 3 years	Karnal	− − +	+ + −	+ + −
10. Berseem growing in the plots for 5 years	Karnal	+ + −	+ + +	+ + +

The results of investigations on bacteriophage both at Lyallpur and Karnal gave a new outlook on this subject. It will be seen from Table III that the plots having berseem stand for 1 to 3 years rarely produce any bacteriophage, while plots having berseem stands for more than 3 years always produce bacteriophage in the nodules of berseem plants. This gave us the solution as to why we did not get any bacteriophage from the berseem plants growing at the Institute Farm for one, two and three years. From both Lyallpur and Karnal results we arrived at a hypothesis that bacteriophage is produced in plots with Berseem plants for three years and more and that no bacteriophage is produced in plots which have got berseem stand less than 3 years old. In support of our hypothesis we have got the work of Demolon and Dunez [1935] in France, and Vandecaveye and Katznelson [1936] in America. A brief summary of their work will not be out of place to mention here.

Demolon and Dunez [1935] succeeded in isolating bacteriophage from the nodules, roots and stems of all alfalfa over one year old on alfalfa sick soils or what they called 'fatigued alfalfa fields' in a number of districts in France, but experienced difficulty or failed in finding it in the roots of alfalfa over 3 years old or in those of old alfalfa plants devoid of nodules. They also isolated bacteriophage from the soil carrying old stands of alfalfa, but not from soils in other crops or carrying young stands of alfalfa. They are of opinion that the presence of this lytic agent in the soil or in plant tissues interferes with normal symbiosis of the nodule bacteria and the host plant. Vandecaveye and Katznelson [1936] isolated a potent lytic principle active against four out of five laboratory stock cultures of Rhizobium moliloti from each of two soils from the Yakina district carrying 3 years old alfalfa stands. They failed to isolate any bacteriophage from the soils carrying alfalfa plants less than 3 years of age. In their case the most common sources of bacteriophage were the soil from fields occupied by alfalfa off and on for many years and 3 and 4 years old stands. Nodulation, on the whole, was poor on plants in fields from whose soils lytic principles were isolated. They could not arrive at any definite conclusion as to bacteriophage being responsible for the reduced yields of alfalfa.

In the light of experience gained at Lyallpur and Karnal and on a line similar to the work of Vandecaveye and Katznelson work was started at Delhi in 1941-42 comprising laboratory, field and pot experiments. In the top block area of Farm berseem was growing since 1937-38 and comprised plots carrying berseem stands for two, three, four and five years. Nodules from these plots were collected during two seasons, 1941-42 and 1942-43 and were examined for the presence of bacteriophage. Two samples were collected—one in January and the other in March. The results of these analyses are given in Tables IV and V.

It will be seen from Tables IV and V that in Delhi soil also bacteriophage was produced in the plots which were bearing berseem stands for three years and more. The soils from the plots having berseem plants for three years and more were

TABLE IV

Production of bacteriophage in Delhi soil (1941-42)

Plot No.	No. of years under berseem	Sample I	Sample II
1. Top Block Plot 2	4 years	+ + +	+ + +
2. Top Block Plot 4	3 years	+ - -	+ - -
3. Top Block Plot 6	2 years	- - -	- - -
4. Middle Block A 1	1 year	- - -	- - -

TABLE V

Production of bacteriophage in Delhi soil (1942-43)

Plot No.	No. of years under berseem	Sample I	Sample II
1. Top Block Plot 2	5 years	+ + +	+ + +
2. Top Block Plot 4	4 years	+ + +	+ + +
3. Top Block Plot 6	3 years	- + +	- + +
4. Middle Block A 1	2 years	Not sown with Berseem	
5. Middle Block A 5	1 year	- - -	- - -

examined for the presence of bacteriophage and were found to contain bacteriophage; while the soils having berseem plants less than 3 years did not contain any bacteriophage. Thus we have confirmed the work of Vandecaveye and Katznelson that bacteriophage does not appear in soils or in nodules of plants growing in the soil for less than three years.

In the light of experience gained by our work at Delhi we will now try to explain why we failed at Pusa to isolate bacteriophage from some of the leguminous plant nodules. At Pusa, bacteriophage was isolated from berseem, sweet pea, karao, dolichos, gram and lucerne. These plants were growing in the same plots at the pot culture area for a number of years. These, by their association with the same soil for a number of years, developed bacteriophage in their nodules, whereas in the case of cowpea, fennugreek, sunn-hemp, *mung*, pea, math and indigo, they were planted one or two times, each in a small plot, simply for the isolation of bacteriophage. The plots varied year after year. This is why we failed to isolate bacteriophage from the nodules of these latter plants as they were not in the same soil for more than one year. In order to test the validity of this statement, pea, fennugreek, lentil, khesari, sweet pea were grown for three years in the same pots each having four separate pots. The nodules of these plants were examined for the presence of bacteriophage. The results are given in Table VI. It will be seen from the table that bacteriophage appeared in pea, sweet pea and began to appear in fennugreek and khesari in the third year of their growth in the same soil. No bacteriophage appeared in the lentil in the third year.

TABLE VI

Produce of bacteriophage in different lagumes

	1941-42		1942-43		1943-44	
	Sample I	Sample II	Sample I	Sample II	Sample I	Sample II
1. Pea	- - -	- - -	- - +	- - -	+ - -	+ + +
2. Fennugreek	- - -	- - -	- - +	- - -	- - -	+ - -
3. Lentil	- - -	- - -	- - -	- - -	- - -	- - -
4. Khesari	- - -	- - -	- - -	- - -	+ - -	+ - -
5. Sweet pea	- - -	- - -	- - -	- - -	+ + -	+ + +

Viability of bacteriophage in soil. Viability of bacteriophage in soil was studied by examining soils containing bacteriophage. In March 1942 eight samples of soil were collected aseptically from Plot A 1 Middle Block growing berseem for the last five years. Five of these soils showed presence of bacteriophage. These soils were kept in the laboratory under the same aseptic condition. They were tested in December 1942 after a period of nine months for the presence of bacteriophage. Almost all of them showed the presence of bacteriophage. It is evident that bacteriophage can survive in the soil for a considerable length of time. The results are given in Table VII.

TABLE VII

Viability of bacteriophage

Soil from Middle Block A 1	March 1943	December 1943
1. Plot B	+++	+++
2. Plot D	+++	+++
3. Plot F	+++	+++
4. Plot G	+++	+++
5. Time of sowing plot	+++	+++

Action of various manures on the production of bacteriophage. In order to study the action of different manures on the production of bacteriophage, samples of nodules from berseem plants were collected from top block plot 1 manurial experiment plots. These plots were under berseem for the last three years. Six plots were selected with different treatments (1) F. Y. M. 80 lb. N, (2) Activated sludge 80 lb. N, (3) Am_2SO_4 80 lb. N, (4) Superphosphate 4 md., (5) Complete minerals, (6) No manure. The nodules were examined for the presence of bacteriophage. The results are tabulated in Table VIII. It will be seen that application of activated sludge, ammonium sulphate and superphosphate favours the production of bacteriophage in the nodules of berseem plants. This experiment has been done for the first time this year and has not been confirmed by subsequent work.

SUMMARY

The root nodules of different leguminous plants were examined for the presence of bacteriophage. While some of the leguminous plants contained bacteriophage in their nodules, others did not show the presence of any. The reason for the presence of bacteriophage has been found to be the association of the plants with same soil for a number of years.

The specificity of bacteriophage has been studied and bacteriophage has been found to be specific for the host plant while some of them cross-inoculate. Viability experiments conducted show that when kept under aseptic condition the bacteriophage remains active for several months. Action of manures on the production of bacteriophage has been studied and it is found that ammonium sulphate, superphosphate and activated sludge favour production of bacteriophage in the root nodules.

TABLE VIII

Presence of bacteriophage

Treatment	23 February 1943	26 March 1943	27 April 1943
1. Plot B F. Y. M. 80 lb. N	− − −	− − −	− − −
2. Plot D Activated sludge 80 lb. N	+ − −	+ − −	+ − −
3. Plot F Am_2SO_4 80 lb. N	++ −	+ − −	++ −
4. Plot G Superphosphate 4 md.	++ −	+ − −	++ −
5. Plot K Complete minerals on the basis of B	− − −	− − −	− − −
6. Plot L No manure	− − −	− − −	− − −

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EFFECT OF ALGAL GROWTH ON THE ACTIVITY OF *AZOTOBACTER* IN RICE SOILS

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(With Plate VI)

The appearance of an abundant growth of algae in submerged rice-fields is a very common phenomenon. Several workers have investigated the rôle of these algae in the transformation of nitrogen in rice soils. De [1936] observed considerable fixation of nitrogen in some rice soils, in which an abundant growth of algae had taken place, after they had been waterlogged and exposed to sunlight for some time. He concluded that the agents responsible for this fixation are certain blue-green algae, and that the part played by *Azotobacter* in this process is relatively unimportant and possibly nil [De, 1939]. Recently Singh [1942] isolated some nitrogen-fixing blue-green algae from rice soils of Bihar and the United Provinces, and observed that these algae are responsible for nitrogen recouperation in these soils. But Chaudhuri [1940], working with rice soils from Faridpur* (Bengal), concluded that algae play a relatively unimportant part in a direct manner in fixing atmospheric nitrogen in the rice-fields, but that the dead algal bodies provide a suitable medium for the growth of *Azotobacter*, on which the latter thrives bringing about considerable fixation of nitrogen. According to him, therefore, it is *Azotobacter*, and not algae, which fixes nitrogen in rice-fields. It is noteworthy, in this connection that several workers have studied the effect of associated growth of pure cultures of algae and *Azotobacter* on the fixation of nitrogen, but obtained no evidence that the former exerts any beneficial effect on the growth and activity of the latter.

Lipman and Teakle [1925] observed small increase in nitrogen (0·5 mg. in 50 c.c. of medium) when *Chlorella* and *Azotobacter* were grown together. The increase, however, is small enough to lie within the experimental error of the nitrogen determination. Allison and Morris [1930] reported that there was no increase in nitrogen fixation when *Azotobacter vinelandii* was grown in association with each of four species of green algae. De [1939] made cultures of two species of blue-green algae with *Azotobacter chroococcum*, and found that the fixation by the associated growth was same as by algae themselves. Recently Stokes [1940] studied the effect of nine strains of green algae on the growth of *Azotobacter chroococcum* and *Azotobacter vinelandii*. *Azotobacter* failed to make appreciable growth in presence of actively growing cultures of algae, or when supplied with their dead cells or the secretion products as the sole source of nitrogen and carbon. From the results the author concluded that the indirect rôle of the majority of soil algae in the fixation of atmospheric nitrogen either is nonexistent or functions to only very small extent.

The present investigation has been undertaken to study the effect of algal growth on the activity of *Azotobacter* in rice soils. It was thought that the knowledge obtained would be useful firstly in deciding whether algae really favour *Azotobacter* growth in rice-fields, and secondly in ascertaining the rôle played by this latter organism in the fixation of nitrogen in these soils.

Experimental

Materials

The algal materials used in this investigation were obtained from Faridpur soil—the same soil as was used by De and Chaudhuri in their experiments. Small portions of algal growths were collected before harvest when the soil was still waterlogged, without disturbing the soil as far as possible. The samples were rapidly dried first in air and then in an oven at 80°C., finely powdered and then preserved for use in the experiment. The nitrogen content of the dry powder was 2·12 per cent. Microscopic examination of the moist growth showed that it was rich in blue-green algae.

Availability of the dead algal bodies as energy materials for Azotobacter

To investigate the availability of the dead algae as a source of energy for *Azotobacter*, 100 c.c. portions of a nitrogen-free solution,§ containing one gram of powdered algal material, were incubated at 30°C. after inoculation with a suspension of soil. A second set of cultures, using mannite (an excellent source of energy for *Azotobacter*) in place of powdered algae, were simultaneously

*De [1939] made an extensive study of this particular soil, from which he isolated some nitrogen-fixing blue-green algae and concluded that these algae directly fix nitrogen in this soil

§K_2HPO_4 — 0·5 gm., $MgSO_4$, $7H_2O$ — 0·2 gm. Na_2MoO_4—0·025 gm., $CaCO_3$—5 gm., $FeCl_3$—0·05 gm. and water 1,000 c.c.

started in order to compare the relative availabilities of these two materials. At the end of 7 and 15 days, duplicate cultures from each treatment were analysed for total nitrogen by Kjeldahl method, there being no nitrate in any of the cultures. The results are shown in Table I.

TABLE I

Nitrogen fixed in culture solutions

(From 26 February to 12 March 1943)

N in mg. in 100 c.c. of the culture solution

Energy material	At start	After 7 days	Fixed in 7 days	After 15 days	Fixed in 15 days
Dead algae	21·5	22·0	0·5	21·5	0·0
Mannite	0·3	9·7	9·4	13·7	13·4

The results in Table I show that the dead algae are not readily available to *Azotobacter* as a source of energy.

Changes in Azotobacter *and total bacterial numbers*

In order to observe the effect of algal growth on the changes in *Azotobacter* and bacterial numbers, 20 gm. portions of soils were distributed in a number of 250 c.c. Erlenmeyer flasks, half of which were previously wrapped with glazed black paper to exclude sunlight. The soils were brought to waterlogged conditions by the addition of either 200 c.c. of distilled water or an equal volume of a nitrogen-free solution, the latter treatment having been found to stimulate algal growth (K_2HPO_4—0·5 gm., $MgSO_4$, $7H_2O$—0·2 gm., $CaSO_4$—0.1 gm., $Ca_3(PO_4)_2$—1.0 gm., $FePO_4$—0·1 gm., water 1,000 c.c.). After the flasks had been plugged with cotton wool and their necks covered with loose rubber caps to prevent rain water, they were all placed in a bath of running water whereupon the whole arrangement was exposed to sunlight. Algae appeared in all flasks in the light, but in none in the dark, i.e. in flasks covered with black paper. At intervals of 15 days, one flask from each treatment was removed for the determination of *Azotobacter* and total bacterial numbers. Total nitrogen was determined after 30 and 60 days.

Azotobacter was determined by a method developed by Jensen [1940]. In this method 0·2 c.c. portions of a soil dilution of 1 : 10 are placed on three sterilized petri dishes containing hardened dextrin-agar medium (medium described earlier, made with 1 per cent dextrin and 2 per cent agar), and evenly spread over by means of a L-shaped glass rod. The excess of water is allowed to evaporate. The plates are incubated at 30°C. for five days after which the colonies are counted. An advantage of this method is that the colonies formed are discreet and very easily distinguishable (Plate VI, fig. 1).

In the present experiment the ratio of soil and water in each flask at start was 1 : 10 (20 gm. of soil in 200 c.c. of water or solution), i.e. the same ratio as is necessary for counting. In the beginning of the experiment, each flask was marked with a glass pencil to indicate the level of the liquid. Before counting the level was brought to this mark by the addition of sterilized water where necessary. Such addition, however, was not generally required as the evaporation of the liquid from the cultures was very little. The flask was then closed with a sterilized rubber cork, vigorously shaken for three minutes and 0·2 c.c. portions of the suspension plated as above.

For bacterial counts, the above suspension (1:10) was further diluted, one c.c. portions of a dilution of 1 : 100,000 being plated with Thornton's [1922] agar. Four Parallel plates were run, which were incubated at 30° C. for seven days, after which the colonies found were counted.

The results of nitrogen determination, and of *Azotobacter* and bacterial counts are shown in Tables II, III and IV respectively.

A reference to Table III shows that the numbers of *Azotobacter* were consistently lower in the light than in the dark, i.e. in presence of algae *Azotobacter* was less abundant. But the results in Table II show that fixation of nitrogen took place only in the flasks in which algal growth had taken place. Thus there is no correlation between nitrogen fixation and *Azotobacter* growth. If the latter organism was really involved in the process, then there would have been fixation (and perhaps in greater amount) in the dark as well where it was present in higher numbers. It will also be remembered that nitrogen fixation is a function of the growth of the nitrogen-fixing organisms, consequently if a particular organism is fixing

TABTE II

Fixation of nitrogen in rice soils

(From 19 May to 17 July 1942)

N in mg. per culture flask

Locality	Days	Soil+Water				Soil+Solution			
		Light		Dark		Light		Dark	
		Total	Fixed	Total	Fixed	Total	Fixed	Total	Fixed
Coimbatore, Madras	0	15·2	..	15·2	..	15·2	..	15·2	..
	30	17·2	2·0	15·0	—0·2	17·2	2·0	14·9	—0·3
	60	18·0	2·8	15·0	—0·2	19·7	4·5	15·0	—0·2
Sabour, Bihar	0	22·0	..	22·0	..	22·0	..	22·0	..
	30	25·0	3·0	21·9	—0·1	26·1	4·1	22·0	0·0
	60	26·1	4·1	21·8	—0·2	28·9	6·9	21·9	—0·1

TABTE III

Effect of algal growth on the changes in Azotobacter *numbers*

(From 19 May to 17 July 1942)

Azotobacter *per 0·1 gm. of dry soil averages are in brackets*

Locality	Days	Soil+Water		Soil+Solution	
		Light	Dark	Light	Dark
Coimbatore, Madras—*p*H=8·40	0	340	340	340	340
		310(333)	310(333)	310(333)	310(333)
		350	350	350	350
	15	135	200	180	240
		120(127)	175(187)	175(178)	255(240)
		125	185	..	225
	30	150	205	200	315
		130(142)	230(212)	205(215)	340(342)
		154	200	245	370
	45	160	300	240	315
		170(160)	325(327)	230(235)	310(330)
		150	355	235	365
	60	105	240	250	360
		105(110)	280(262)	245(253)	320(335)
		120	265	265	325
Sabour, Bihar—*p*H=8·30	0	500	500	500	500
		520(500)	520(500)	520(500)	520(500)
		480	480	480	480
	15	235	250	475	500
		250(242)	265(252)	450(452)	500(513)
		240	240	430	540
	30	300	450	470	600
		335(315)	480(478)	485(492)	630(600)
		310	505	520	570
	45	300	590	455	645
		360(320)	530(552)	425(440)	620(650)
		300	535	440	685
	60	120	600	385	640
		115(125)	585(600)	380(390)	630(625)
		140	615	405	605

nitrogen in a certain environment, its number should show enormous increase at some stage at least during the process. But as seen in Table II, in none of the soils and at no stage of the experiment, did *Azotobacter* show any tendency to multiply, showing thereby that the conditions occurring in the cultures were never suitable for its growth. All these facts go to prove that *Azotobacter* does not play any part in the fixation of nitrogen in rice soils under waterlogged condiions.

TABLE IV

Effect of algal growth on the changes in bacterial numbers

(From 19 May to 17 July 1942)

Bacteria as millions per gm. of dry soil; average of 4 plates

Locality	Days	Soil+Water		Soil+Solution	
		Light	Dark	Light	Dark
Coimbatore, Madras . .	0	3·60	3·60	3·60	3·60
	15	12·10	14·02	12·52	14·86
	30	16·40	21·50	17·20	21·00
	45	11·60	16·60	12·70	17·50
	60	5·65	13·75	12·00	17·33
Sabour, Bihar . . .	0	5·10	5·10	5·10	5·10
	15	11 55	15·77	13·60	16·30
	30	13·13	18·90	14·25	14·55
	45	8·73	16·05	7·80	12·45
	60	8·60	13·00	7·90	11·25

Like *Azotobacter*, bacterial numbers were also less in presence of algae. It was also suspected that some products, toxic to *Azotobacter* and other bacteria, might have been formed in the soil as a result of algal growth. The following experiment was undertaken to test this possibility.

Two equal amounts of a rice soil were brought to waterlogged conditions by the addition of same volume of distilled water and then exposed to sunlight, after having covered one with black paper. As observed in other experiment, algae appeared only in the soil kept in the light. After two months, the supernatant water from each was carefully siphoned off and filtered. Chemical analysis showed that the filtrates contained very little N (1 p.p.m.) and no phosphate. Using these two filtrates in place of distilled water, two dextrin-agar media were prepared according to the formula given previously. Equal volumes of the melted media were hardened in petri dishes of same size, and by means of a glass pencil a straight line, 1 cm. in length, was drawn on the bottom side of the lower half of each petri dish. The plates were inoculated by streaking a loopful of a suspension of *Azotobacter* in water over the medium just above and from end to end of the line drawn. In this way the conditions of inoculation were standardized —the number of cells in each inoculum and the amounts of nutrients available to them being kept constant as far as possible.

Plate VI, fig. 2 shows the growth of *Azotobacter* in the two media after 5 days' incubation at 30°C. The growth is apparently considerably less in the medium made with the water from soil containg algal growth. In fact, when freshly prepared media were used, the difference was more marked than as shown in Plate VI, fig. 2. It will be seen that the two plate cultures were made under exactly similar conditions, except that the samples of water used in the preparation of the media were obtained from different sources. The difference in growth must, therefore, be due to the difference in qualities of the two water samples. Evidently the sample obtained from soil with algal growth had contained something which inhibited the

growth of *Azotobacter*. This explains why this organism was less abundant in presence of algae.

Decomposition of dead algal bodies

After harvest rice soils are allowed to dry up when the algae which had appeared during the previous waterlogged period die, forming a dry scum over the soil surface. The material ultimately decomposes in the soil, the conditions of which remain very suitable for bacterial activity, particularly during the few months in winter. The average temperature lies between 25-30°C., and there remains in the soil sufficient moisture to favour the growth of the micro-organisms.

In order to find whether the decomposition of dead algae in soil under dry conditions leads to fixation of nitrogen, 100 gm. portions of soils were distributed in two glass dishes, one of which remained untreated and the other received an addition of 2·5 gm. of powdered algal bodies. The moisture contents of the soils were adjusted to 15 per cent on dry basis and were maintained at this value throughout the experiment by periodic additions of water. The dishes were placed in an incubator at 30°C., and the soils were daily stirred to facilitate aeration. At intervals, soil samples were withdrawn for determination of total nitrogen and for *Azotobacter* and bacterial counts. Total nitrogen was determined by the modified Kjeldahl wet method, there being no nitrate accumulation in the soils. *Azotobacter* and bacteria were counted by the methods already described.

Results of nitrogen determination are given in Table V and *Azotobacter* and bacterial counts in Tables VI and VII respectively.

TABLE V

Decomposition of dead algal bodies on nitrogen fixation

(From 26 February to 26 April 1943)

N in mg. per 100 gm. dry soil

Locality	Treatments	At start	After 15 days	After 30 days	After 45 days	After 60 days	Fixed in 60 days
Faridpur, Bengal	No treatment	47·0	48·0	46·5	47·0	47·0	0·0
	Algae*	100·0	101·0	100·0	97·5	96·0	—4·0
Coimbatore, Madras	No treatment	82·0	..	81·0	82·0	81·5	—0·5
	Algae	135·0	..	135·0	132·0	130·5	—4·5
Sabour, Bihar	No treatment	116·5	..	116·8	116·0	117·0	+0·5
	Algae	169·5	..	170·0	167·0	165·0	—4·5

*N added with 2·5 gm. of powdered algae = 53·0 mg.

Results in Table V show that, instead of there being any fixation of nitrogen, considerable loss of this element took place from every soil treated with powdered algae. It is not known how this loss was brought about, but this is a common observation that when soil is treated with materials rich in nitrogen, some loss of this element generally takes place. At any rate, there is no evidence that nitrogen-fixation takes place in the rice soils as a result of the decomposition of dead algae.

This conclusion is also supported by *Azotobacter* counts which showed no sign of multiplication (Table VI). But the total bacteria multiplied enormously both in the control as well as in the treated soil—considerably more in the latter (Table VII). This shows that the addition of algae had made the soil more favourable for bacterial growth; this observation further suggests that the 'inhibitory substances' formed in the waterlogged soil probably did not survive under dry conditions.

The foregoing evidences are definitely against the view that *Azotobacter* fixes nitrogen in rice soils, utilizing the dead algal bodies as energy source. Chaudhuri [1940] came to such conclusion with respect to rice soils of Bengal, but he has given no data and practically no evidence in support of his statements.

In this investigation only the rôle played by *Azotobacter chroococcum* has been considered. This is the most commonly occurring species which has been found to be widely distributed in Indian

TABLE VI

Decomposition of dead algal bodies on Azotobacter *numbers*

(From 26 February to 6 April 1943)

Azotobacter *per* **0·1** *gm. of dry soil averages are in brackets*

Locality	Days	Treatments	
		No treatment	Algae
Coimbatore, Madras *p*H=8·40	0	227	227
		205(223)	205 (223)
		238	238
	10	205	181
		228(209)	170(181)
		194	192
	20	279	205
		302(283)	194(198)
		267	194
	30	219	148
		184(204)	160(152)
		209	148
	40	270	212
		290 (280)	198(199)
		280	187
Sabour, Bihar *p*H=8·30	0	393	393
		424(403)	424(403)
		393	393
	10	765	620
		765(743)	630(616)
		700	597
	20	640	410
		700(660)	390(400)
		640	400
	30	1200	607
		1150(1150)	607(598)
		1100	580
	40	1370	680
		1310(1320)	690(670)
		1280	640
Faridpur, Bengal *p*H=8·35	0	20	20
		20(20)	20(20)
		20	20
	10	10	10
		10(10)	0(3)
		10	0
	20	137	0
		126(126)	0(0)
		114	0
	30	239	10
		219(229)	10(10)
		228	10
	40	260	10
		236(252)	10(7)
		260	0

rice soils. The recently discovered *Azotobacter indicum* [Starkey and De, 1939] has been found only in a few soils, and as regards other species of this genus, their occurrence in Indian rice soils has not been demonstrated. Besides *Azotobacter*, *Clostridium pasteurianum* and other anaerobic nitrogen-fixing bacteria might also play an important rôle in the process particularly during the waterlogging periods when the conditions of the soil remain partly anaerobic. This possibility is at present under investigation, the results of which will be published in due course.

TABLE VII

Decomposition of dead algal bodies on bacterial numbers

(From 26 February to 6 April 1943)

Bacteria as millions per gm. of dry soil; average of 4 plates

Locality	Days	Treatments	
		No treatment	Algae
Coimbatore, Madras	0	3·5	3·5
	10	7·0	21·0
	20	155·2	225·0
	30	552·5	765·0
	40	602·0	1040·0
Sabour, Bihar	0	6·0	6·0
	10	62·1	109·0
	20	179·0	225·7
	30	370·0	506·2
	40	628·3	930·0
Faridpur, Bengal	0	1·7	1·7
	10	12·3	38·7
	20	73·0	107·8
	30	343·7	592·0
	40	421·6	702·0

SUMMARY

There was no fixation of nitrogen when a culture solution, made with the necessary nitrogen-free nutrients and dead algal bodies from a rice field as the source of energy, was inoculated with a suspension of soil, showing that these algae do not serve as energy materials for nitrogen fixation.

Periodic estimations of *Azotobacter*, and total bacteria in waterlogged rice soils, kept in the light (algae present) and also in the dark (algae absent), showed that the numbers of these organisms were consistently less in the former, in spite of the fact that in these cultures nitrogen fixation had taken place. The results further showed that *Azotobacter* failed to multiply in either treatment. Evidence was obtained that some materials toxic to *Azotobacter* are formed in rice soils under waterlogged conditions as a result of algal growth.

Determinations of total nitrogen at intervals in soils, treated with and without dead algae and kept under optimum conditions of temperature and moisture, showed considerable loss from the former. Here again *Azotobacter* was relatively less in the soil treated with algae and there was

FIG. 1. Plate Counts of *Azotobacter* on dextrin-agar. Incubated 5 days at 30°C.

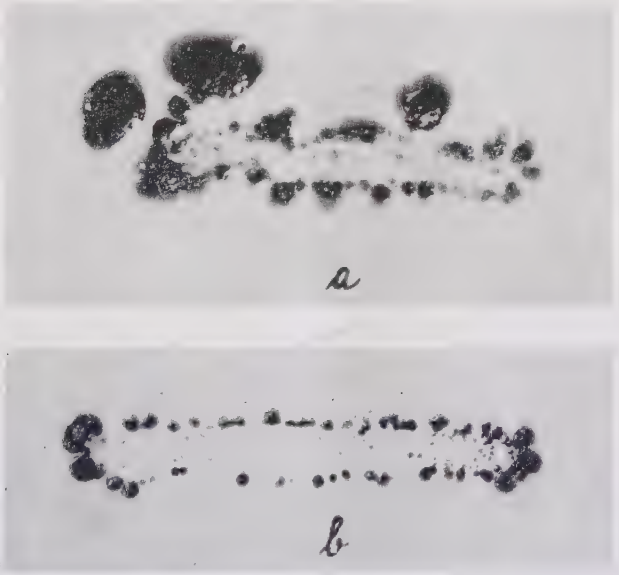

FIG. 2. *Azotobacter* growth on dextrin-agar plate. Incubated 5 days at 30°C.

a—Medium made with water from a soil without algal growth

b—Medium made with water from the same soil with algal growth

FIG. 1. Bacterial leaf-spot on betel leaf, early stage

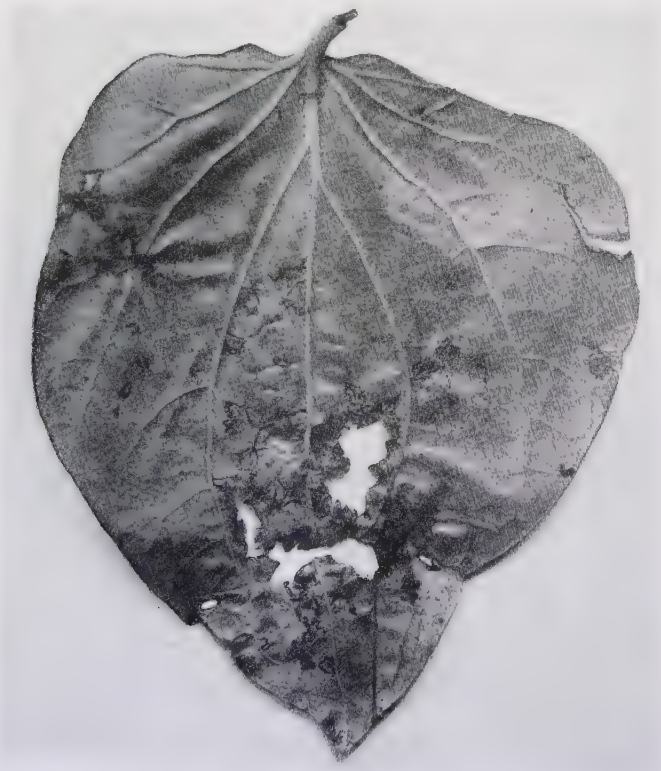

FIG. 2. Bacterial leaf-spot on betel leaf, advanced stage

no sign of its multiplication in either treatment. Total bacteria, on the other hand, showed increase in both treatments—much more in presence of algae. It is presumed that the failure of *Azotobacter* to multiply in these soils is partly due to competition with total bacteria for the supply of available nutrients.

From the evidences obtained it is concluded that the algal growths in the rice-fields do not provide a suitable medium for the growth of *Azotobacter*, and further that this latter organism does not bring about any nitrogen-fixation when using the dead algal bodies as source of energy.

ACKNOWLEDGEMENTS

In conclusion the author wishes to express his gratitude to Dr P. K. De for his kind guidance and criticisms throughout the progress of this investigation and to the Imperial Council of Agricultural Research for financial help.

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BACTERIAL LEAF-SPOT OF *PIPER BETLE*

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(With Plate VII)

BACTERIAL leaf-spot disease of *pan* (*Piper betle*) has been known to exist in the Central Provinces and Berar for more than a century. This disease, called *kinrog* by local *pan* growers, attracted little attention till 1933 when a survey was undertaken to determine its intensity at different *pan*-growing centres. The disease has been reported from Ramtek, Pauni, Sohagpur, Mandla, Timarni, Anjangaon, Saugor, Damoh, Bhandara, Drug and several other places in the province. It has been observed that all the varieties of *pan* that are cultivated in this province, viz. *kapuri, bangla, kakher* and *gangeri* are susceptible to it to varying degrees of intensity and the annual loss caused by it is considerable.

Hutchinson [1924] described a bacterial disease on stem of betel vine in Bengal and in 1925 he reported that the bacterium isolated from the diseased material failed to give conclusive results, though, later in the year, one or two individuals showed symptoms of the disease. Raghunathan [1926] was the first to report the bacterial leaf-spot of betel, which was recorded in Ceylon in 1896. He mentions that from 1921 onwards the disease did considerable damage to cultivations in several localities in the low country and was most prevalent during the monsoon. The cause of the disease was attributed to a bacterium which he named as *Bacterium betle*. The symptoms of the disease have been mentioned. As control measures, destruction of all diseased material six months of rotation with vegetables and general sanitation have been recommended. Later Raghunathan [1928] published a fuller account of his investigations. He gave a technical description of the organism, whose group number according to the chart of the Society of American Bacteriologists, was 2,11,00,00,514. Nirula [1931] described a bacterial disease of betel leaves during storage. Symptoms, methods of infection and the characters of the pathogen show that the disease as described by him is quite different from the one which is being described here. Park [1934] has redescribed the symptoms, the methods of transmission and of control of the leaf spot caused in Ceylon by *Bacterium betle*.

The earliest symptom of the disease is the presence of minute transluscent specks in-between the veins on the underside of the leaves. With in two to three days these transluscent specks

are visible on the upper surface as circular or angular brown to dark-brown spots, surrounded by yellow coloured zones (Plate VII, fig. 1). On the underside of the leaves the areas corresponding to the yellow zones are somewhat water-soaked in appearance. When atmospheric conditions are dry the spots are localized and minute, measuring 1 to 5 mm. in diameter ; but with the advent of humid and wet weather, they get considerably enlarged and measure up to 1 cm. across. In old and large spots the central portion often cracks or falls off (Plate VII, fig. 2). The number of spots on a leaf varies according to the prevailing weather conditions. When the atmosphere is humid the spots often coalesce, causing larger patches of dead areas on the leaf blade. Such leaves gradually loose their lustre, become flaccid turn pale and finally fall off. A gummy substance full of bacteria oozes out during wet weather and gets deposited on the underside of an infected leaf. The disease has never been observed to occur on stems, petioles, or roots.

In a *pan* garden the disease is mostly evident on leaves which are near or touching the soil surface. During dry weather the infection is confined to a height of 1 ft. of the vines but during wet seasons it extends to a height of 3 ft. due to the splashes of rain water. Mites have been observed to carry the infection from leaf to leaf. Whenever these insects appear in virulent form the disease spreads even up to a height of 5 ft. of the vines. Rain water and insects are the chief carriers. The disease has been observed to be more prevalent in gardens with heavy soils than in gardens with light soils. *Pan* vines cultivated in low-lying and waterlogged areas are the greatest sufferers.

At Mandla the incidence of the disease on *bangla* variety of *pan* was recorded monthly from three well-drained high-lying and three low-lying waterlogged gardens for a period of 12 months. The data show that the incidence of the disease was more than double, specially from July to December, in low-lying waterlogged gardens than in high-lying well-drained ones. The average percentage of infection for 12 months was 12·8 in the former and 5·9 in the latter. In both the cases the disease was more pronounced during wet weather and scarce during dry months with scanty or little rainfall.

Experiments very much similar to that of Mandla were carried out at Ramtek for three years, 1941-43. Here the incidence of the disease was recorded on two varieties of betel leaves, viz. *bangla* and *kapuri*, the latter proving more susceptible to the disease than the former. Temperature and humidity played important röles on the incidence of the disease. High atmospheric temperature with little or scanty rainfall definitely retarded the incidence of the disease while, during wet months of July, August and September, the infection was very high on both the varieties.

Pure cultures of the pathogenic bacteria were isolated. On glucose agar medium honey-yellow coloured colonies appeared within three days of inoculations. The individual organisms are cylindrical in shape with the ends slightly rounded and measure $1 \cdot 5\text{-}2 \cdot 5 \times 0 \cdot 5\mu$. They are either solitary or in chains of three to four cells, light honey-yellow in colour, non-motile and without spores or capsules. Pathogenic and cultural characters of the organism indicate that it is the same bacterium (*Bacterium betle*) as described by Raghunathan [1926], causing leaf-spot in Ceylon.

Healthy roots, stems, petioles and leaves of betel vines were artificially inoculated with pure cultures of the organism. The disease could be readily transferred to leaves when kept under moist condition. According to the age of the leaves the symptoms appeared within one to four days. Old and mature leaves took up the inoculation much earlier than young and immature ones. Infection was more pronounced on the lower side of the leaves than on the upper side which indicated that the organism made its entry to the host tissue through stomata which are abundant on the lower sides. Unless and until the upper sides of the leaves were injured they did not get infected. The infection on the lower surface appeared within 3 days ; spots were numerous and close together. Invariably in every case inoculations with cultures older than two weeks proved unsuccessful. It was also observed that if the inoculum was placed at the cut end of a stem, the infection advanced at the most to 1 cm. where the tissues turned dark brown and the cells collapsed. In no case was the infection observed to advance to any appreciable length in the cuttings. Healthy roots, stems and petioles when wounded and inoculated gave negative results.

Several experiments were conducted to work out suitable measures of control for the disease. At Timarni spraying trials with Bordeaux mixture (2:2:50), Bouisol (3·5 lb. in 100 gallons of water) and Sulsol (5 lb. in 100 gallons of water) were conducted to find their effects on the incidence of the disease. One gallon of each of the fungicide was sprayed on 75 ft. length of the *pan* rows. Four varieties, viz. *bangla*, *kapuri*, *kakher* and *gangeri*, were under trial with the above three fungicides. Only one spraying could be given during August but the results when observed after two months showed that there was 10 per cent disease in unsprayed vines, eight per cent in Sulsol, four per cent in Bouisol and only one per cent in Bordeaux mixture sprayed vines. *Kapuri* was the most

TABLE I

Effect of fungicides on the incidence of leaf-spot disease on large leaves (Bade pan)

Plot No.	Treatment of soil	Treatment of vines	March Air temp.—80·5 Rainfall—0·52			April Air temp.—87·0 Rainfall—0·59			May Air temp.—94·8 Rainfall—0·64			June Air temp.—84·6 Rainfall—13·12			July Air temp.—81·0 Rainfall—14·45			August Air temp.—79·6 Rainfall—13·52			September Air temp.—80·1 Rainfall—7·56			October Air temp.—78·6 Rainfall—7·03			Grand total for the year*		
			Total number of picked leaves	Number of infected leaves	Per cent of infection	Total number of picked leaves	Number of infected leaves	Per cent of infection	Total number of picked leaves	Number of infected leaves	Per cent of infection	Total number of picked leaves	Number of infected leaves	Per-cent of infection	Total number of picked leaves	Number of infected leaves	Per cent of infection	Total number of picked leaves	Number of infected leaves	Per cent of infection	Total number of picked leaves	Number of infected leaves	Per-cent of infection	Total number of picked leaves	Number of infected leaves	Per-cent of infection	Total number of picked leaves	Number of infected leaves	Per cent of infection
1	No treatment . . .	No treatment . . .	9,490	989	10·4	11,436	497	3·4	15,567	318	2·0	14,322	2,986	20·8	36,185	12,635	34·9	38,387	14,171	36·9	30,652	5.814	18·0	16,848	1,989	11·8	1,72,887	39,399	22·76
2	Bordeaux mixture (2 : 2 : 50), 1 gallon for 6 ft.	No treatment . . .	12,593	898	7·1	11,017	128	1·1	16,223	176	1·0	15,394	1,543	10·0	51,001	12,750	24·9	60,911	12,789	20·9	59,713	8,758	14·6	55.413	2,571	4·6	2,82,265	39,612	14·0
3	Ditto.	Bordeaux mixture (2 : 2 : 50), 1 gallon for 75 ft.	11,989	277	2·3	10,240	32	0·3	15,580	37	0·2	16,452	212	1·2	47,927	2,422	5·0	61,325	1,226	1·8	59,918	1,989	3·3	57,769	31	0·5	2,81,200	6,507	2·3
4	Ditto.	Bouisol (5 lb. in 100 gallons), 1 gallon for 75 ft.	9,633	165	1·7	12,133	27	0·2	14,972	42	0·2	15,913	249	1·5	48.932	3,398	6·0	59,221	1,812	3·0	61,271	2,448	3·9	54,363	401	0·7	2,76,438	8,542	3·0
5	Ditto.	Sulsol (5 lb. in 100 gallons), 1 gallon for 75 ft.	11,807	948	8·0	10,712	113	1·0	15,719	33	0·2	14,057	1,072	7·1	48,761	11,902	24·4	61,017	10,980	17·9	59,676	8,040	13·5	56,591	2,286	4·0	2,79,240	35,374	12·6

*For the first four months of the plantation no pick-ng is carr (dout

TABLE II

Effect of fungicides on the incidence of leaf-spot disease on small leaves (Khilli)

Plot No.	Treatment of soil	Treatment of vines	March Air temp.—80·5 Rainfall—0·52			April Air temp.—87·0 Rainfall—0·59			May Air temp.—94·8 Rainfall—0·64			June Air temp.—84·6 Rainfall—13·12			July Air temp.—81·0 Rainfall—14·45			August Air temp.—79·6 Rainfall—13·52			September Air temp.—80·1 Rainfall—7·56			October Air temp.—78·6 Rainfall—7·03			Grand total for the year*		
			Total number of picked leaves	Number of infected leaves	Per cent of infection	Total number of picked leaves	Number of infected leaves	Per cent of infection	Total number of picked leaves	Number of infected leaves	Per cent of infection	Total number of picked leaves	Number of infected leaves	Per cent of infection	Total number of picked leaves	Number of infected leaves	Per cent of infection	Total number of picked leaves	Number of infected leaves	Per cent of infection	Total number of picked leaves	Number of infected leaves	Per cent of infection	Total number of picked leaves	Number of infected leaves	Per cent of infection	Total number of picked leaves	Number of infected leaves	Per cent of infection
1	No treatment . . .	No treatment . . .	5,978	708	11·8	5,337	159	2·9	7,908	117	2·7	6,759	1,072	15·8	20,343	5,987	29·4	19,184	6,494	33·6	17,106	2,007	11·7	9,231	744	8·0	91,846	17,288	18·8
2	Bordeaux mixture (2 : 2 : 50), 1 gallon for 6 ft.	No treatment . . .	4,383	301	6·8	5,095	25	0·4	8,002	160	1·9	7,624	988	11·6	25,912	4,032	15·5	33,712	5,840	17·3	35,119	5,021	14·2	25,713	780	3·0	1,45,560	17,147	11·7
3	Ditto.	Bordeaux mixture (2 : 2 : 50), 1 gallon for 75 ft.	5,079	76	1·4	5,170	12	0·2	7,235	36	0·4	6,902	132	1·9	23,221	598	2·5	33,315	432	1·2	31,712	1,200	3·7	28,919	89	0·3	1,41,553	2,575	1·7
4	Ditto.	Bouisol (5 lb. in 100 gallons), 1 gallon for 75 ft.	4,547	79	1·7	6,706	17	0·2	8,101	40	0·4	7,121	179	2·5	26,715	487	1·8	32,122	1,105	3·4	35,923	2,163	6·0	29,911	307	1·0	1,51,146	4,377	2·8
5	Ditto.	Sulsol (5 lb. in 100 gallons), 1 gallon for 75 ft.	6,042	480	7·9	5,603	67	1·1	7,743	39	0·5	7,619	836	10·9	26,219	5,132	19·13	34,017	6,821	20·0	32,211	3,342	10·3	27,733	1,280	4·6	1,47,187	17,997	12·2

* For the first four months of the plantation no picking is carried out

susceptible variety. Though Bordeaux mixture proved best of all the tried fungicides yet the *pan* growers preferred Bouisol as it does not leave any trace of the chemical on the leaves whereas in the former they become unsightly and can only be marketed after thorough washing and cleaning with water which involved extra labour and time.

The above experiment was later repeated at Ramtek with only one variety of *pan*, viz. *kapuri*. An area of $\frac{1}{3}$ acre was taken for this trial which had 50 rows of betel-vines. This area was divided into five equal plots, each with 10 rows, and each row being 160 ft. in length. With the exception of plot No. 1, the rest were irrigated with 2:2:50 strength Bordeaux mixture before planting the cuttings. Vines in plots No. 1 and 2 were not given any spraying while 3, 4 and 5 were sprayed with Bordeaux mixture (2:2:50), Bouisol (2·5:50) and Sulsol (2·5:50) respectively at the rate of one gallon for 75 linear lengths of the vines. Irrigation of the soil with Bordeaux mixture (2:2:50) was carried out in November, a week prior to planting, while the spraying was done after three months of it. Subsequently both the treatments were carried out once every two months. The leaves were picked every month and graded into *bade pan* (large) and *khilli* (small) leaves. The results are given in Tables I and II.

In these experiments also the best results were obtained where the soil and the vines were treated with Bordeaux mixture (2:2:50), closely followed by Bouisol (2·5:50). Spraying the vines with Sulsol did not give satisfactory results. Where the soil was treated with Bordeaux mixture and no spraying was given (Plot 2), the incidence of the disease was slightly less than the untreated plot (No. 1) as the infection from soil was minimized. However, this soil treatment alone of plot No. 2 was not at all effective in checking the progress of the disease. It will be further noticed that here again the disease was at its highest during the months of July and August, a period of high humidity and low temperature.

Repeated trials have shown that this disease can be successfully controlled if the following measures are adopted by *pan* growers :

1. The vines when taken off the stake and lowered should be so retied that the lower most leaves are at least one foot above the soil surface.

2. The diseased leaves should be systematically and regularly picked and destroyed by burning.

3. The irrigation channels should be so constructed that no waterlogged areas are created in the garden.

4. In case mites appear in a garden, the infected and neighbouring vines should be sprayed with a solution of fish-oil rosin soap (4 oz.) and nicotine sulphate (1 oz.) or tobacco decoction, in 6 gallons of water. Dusting the vines with finely powdered sulphur (200 mesh) will also remove the mite trouble.

5. One week before planting the cuttings the soil should be irrigated with 4:4:50 strength* Bordeaux mixture and subsequently it should be treated every two months at the rate of 1 gallon for 12 linear ft.

6. The vines should be sprayed with 2:2:50 strength Bordeaux mixture every two months, 1 gallon for 75 ft. length of the vines.

This treatment will also effectively control anthracnose (*Colletotrichum* sp. and *Gloeosporium* sp.) and leaf-rot (*Phytophthora parasitica* Dast. var. *piperina* Dast.) diseases.

The cost of six sprayings per annum per acre will amount to Rs. 13, Rs. 82 and Rs. 82 for Bordeaux mixture, Bouisol and Sulsol, respectively at pre-war rates. The savings over untreated gardens per acre per annum was Rs. 506 for Bordeaux mixture, Rs. 411 for Bouisol and Rs. 162 for Sulsol. These results definitely show that the *pan* growers will be amply rewarded if they take up the recommended treatments.

By spraying the leaves with Bordeaux mixture unsightly stains are left over to which the *pan* growers strongly object. This defect can easily be removed if the sprayed leaves are first dipped in a 2 per cent solution of ammonium chloride for an hour and then washed with water. The extra cost involved in this way will hardly come to Rs. 100 per annum per acre, which will be well within the profit scope of the growers.

It was apprehended that the Bordeaux mixture sprayed leaves, when consumed, might prove harmful. The Agricultural Chemist to Government, Central Provinces and Berar, was therefore requested to conduct the necessary analysis and samples were sent over to him. On the results of the analysis he remarks, ' the maximum amount of copper present in unwashed selected sprayed leaves was 2·45 grains per lb. whereas when the same were washed, the quantity present was 1·11 grains per lb. and the average sample of unwashed leaves from the sprayed vines contained 0·82 grain per lb. Taking an average that 200 leaves weighed one lb. and assuming that the maximum consumption per day per person is 20 betel leaves then the maximum amount of copper taken per day per person, if the leaves are unwashed and if all of them show the spots of dried Bordeaux mixture, would come to 0·24 grain whereas if the leaves are washed—as is the usual practice—the quantity of copper taken per person per day will come to only 0·1 grain, both of which

*Instead of 2:2:50 strength 4:4:50 Bordeaux mixture is recommended as it has been found that this strength will control the bacterial leaf-spot and as well as foot-rot disease

are lower than the maximum that can be taken without causing any injury'. On this report and as well as by every day observation Bordeaux mixture can be safely recommended for spraying the vines as the leaves are always consumed after washing.

Summary

The annual loss to *pan* growers due to bacterial leaf-spot disease in the Central Provinces and Berar is considerable.

Symptoms of the disease are described. The disease is more prevalent during wet weather, in heavy soils and in low-lying waterlogged gardens.

Kapuri variety of *pan* is more susceptible to the disease than *bangla, kakher* or *gangeri* varieties.

The pathogenic organism is similar to *Bacterium Betle* Raghunathan in its cultural and pathogenic behaviour.

Neither by artificial inoculations nor under natural conditions has the bacterium been observed to cause infection to roots, stems or petioles.

The bacterium enters the leaves either through stomata or injured surface.

Irrigating the gardens with 4:4:50 Bordeaux mixture one week prior to planting and spraying the vines with 2:2:50 strength Bordeaux mixture every two months successfully controlled the disease and the cost of treatment will amply reward the growers financially.

Acknowledgement

We wish to express our sincere thanks to Rao Bahadur Dr D. V. Bal, Agricultural Chemist to Government, Central Provinces and Berar, for the analysis of Bordeaux mixture stained leaves.

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STUDIES ON THE VIRUS DISEASES OF POTATOES IN INDIA

I. OCCURRENCE OF SOLANUM VIRUS I

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(With Plates VIII and IX)

During analytical work of viruses of potatoes growing in the field and in the insect proof house at the Imperial Agricultural Research Institute, New Delhi, exhibiting various types of symptoms, e.g. different types of mosaic and necroses, a number of cultures of *Solanum virus* I. Orton [Smith, 1937] were recovered from plants of several potato varieties. A culture of the same virus was also obtained from a plant of *Majestic* variety, the leaves of which showed negligible mottle accompanied with severe veinal necroses. The necroses seemed to have appeared on the undersurface in the first instance and later appeared on the upper surface of the leaves. The plant was stunted and its growth in general was poor. The majority of plants of this variety in this particular field showed mild veinal necroses leading to acropetal necrosis. The plant under reference differed from the others in the fact that it showed intense veinal necroses without its subsequent development into acropetal necrosis. The seed tubers of *Majestic* variety had been obtained from Divisional Superintendent of Agriculture, Bhowali, United Provinces.

Some cultures appeared to be more virulent than others, as determined by the intensity of symptoms produced on the differential hosts. The culture from the plant of *Majestic* variety appeared to be of moderate virulence and experiments were conducted to study its properties. These are reported in this paper.

Material and method

Preliminary analyses were carried out by transferring the extracts of infected potato plants to sets of differentials such as *Datura stramonium L.*, *Nicotiana tabacum* L. var. *White Burley*, *Nicotiana glutinosa* L., *Nicotiana tabacum* L. var. *German Samsun*, *Nicotiana rustica* L., *Nicotiana sylvestris* Spegaz and Comes, *Solanum nodiflorum* Jack., *Capsicum annuum* L., *Petunia hybrida* Vilm., *Lycopersicum esculentum* Mill. var. *Sutton's Early Market*. The reactions on these hosts gave an indication with regard to the presence or otherwise of virus. During this analysis the potato plant of *Majestic* variety proved to have been infected by a mixture of viruses. Solanum virus I was separated by passage through *Datura stramonium* which was found to be resistant to the other virus constituent. The second constituent was transmitted by juice inoculations to *Petunia hybrida* on which it showed vein clearing in early stages and later exhibited greening along the veins. On *Solanum nodiflorum* it developed veinal mosaic, showing that the constituent was *Solanum* Virus 2. Orton.

The culture of the virus thus isolated and purified was maintained on young tobacco plants of variety *White Burley* raised in sterile soil under insect proof conditions. Later on, transfers were made to a batch of young plants periodically in order to provide an inoculum from a stock of freshly infected plants throughout the course of the investigation. All plants were grown in 3 in.-4 in. clay pots. Standard extract for inoculation purposes was prepared by crushing to fine pulp in pestle and mortar a definite quantity of young infected leaves which had previously been washed several times in distilled water and dried in folds of filter paper, adding a small quantity of sterilized distilled water from time to time. To every gram of leafy material, 1 c.c. of water was added. This was then pressed through muslin by hand. The extract thus obtained was either used as such or filtered through filter paper as considered necessary for each experiment.

Inoculations were usually carried out on young tobacco plants of variety *White Burley* by smearing the leaf with a piece of sterilized absorbent cotton wool dipped in the standard extract of the culture to which a little carborundum powder had previously been added. Occasionally plants of *Nicotiana tabacum* were also used as indicators but for any one experiment the same variety of plants was used. This method usually gave 100 per cent infections. Checks were similarly treated except that extract from healthy plants was used as inoculum. Every care was taken to maintain aseptic conditions and all the apparatus used was sterilized in accordance with the needs of each experiment. Hands were thoroughly washed with lysol water and soap between each experiment.

The plants, as a rule, were inoculated when they developed the first 2-4 leaves and for any one experiment plants of the same age were employed so that the results could be strictly comparable. The inoculated plants were always kept under observation for at least 15 days.

The virus which has been described is defined by its reactions on the solanaceous plants. The reactions described are those observed during the months of December-March at Delhi in the presence of sufficient light and humidity.

Reactions on differential hosts

Nicotiana tabacum var. *White Burley*. Infection of this host is rapid and the symptoms often begin to appear two days after inoculation. The inoculated leaves first show faint mottle and later develop circular chlorotic spots which gradually become very distinct. Some of these spots may join and form a large chlorotic area. The infection soon becomes systemic and the younger leaves show small prominent chlorotic spots which are circular and some of which tend to be concentric. In youngest leaves the chlorotic spots sometimes develop necrotic centres. Later on all the old and young leaves show pronounced chlorotic ring spots. The symptoms in general are well pronounced (Plate VIII, fig. 1).

On variety *German Samsun* the first symptoms of infection are observed on the inoculated leaves about 5 to 6 days after inoculation. The leaves show either regular mottling of light with dark green or circular chlorotic spots. The symptoms are not marked.

The symptoms produced on *Nicotiana tabacum* are almost the same as produced on var. *White Burley*. Circular chlorotic spots appear on leaves 4-5 days after inoculation. On older leaves the spots are often well scattered over the whole surface and are in the form of characteristic rings. Later on the symptoms get more pronounced. (Plate VIII, fig. 2.)

Nicotiana glutinosa. Symptoms on inoculated leaves usually appear about six days after inoculation. Younger leaves show chlorotic spots

alongside the veins or on the veins or both. These spots later become very distinct and quite often join to form large irregular chlorotic areas. Older leaves also sometimes develop large circular chlorotic spots and some of these spots may develop necrotic centres.

Nicotiana rustica. Regular circular chlorotic spots begin to appear on inoculated leaves about 3-4 days after inoculation. These are surrounded by light green margin and often take the shape of concentric spots. The infection becomes systemic and in a few days typical concentric spots are observed on almost all the leaves (Plate VIII, fig. 3).

Nicotiana sylvestris. The symptoms begin to show a bout six days after inoculation. The first obvious symptom is the occurrence of light chlorotic spots on the inoculated leaves. Later when the infection becomes systemic the younger leaves also exhibit similar symptoms. The chlorotic spots are not concentric.

Datura stramonium. In 4-5 days after inoculation the leaves first exhibit light mottling which develops into distinct circular chlorotic spots. These spots cover the entire surface of the leaf. About four days later vein-clearing is observed on the younger leaves. At the same time some of the middle leaves also show vein-clearing near the basal region which gradually spreads upwards and eventually covers the entire surface. Sometimes chlorotic spots are also observed on the young leaves. Young leaves which show vein-clearing later develop severe chlorosis between the veins along which a deep green portion persists. This symptom is more pronounced at the basal portion of leaves. Older leaves continue to show faint chlorotic or pale spots. Some leaves even develop necrotic lesions (Plate IX, figs. 1-A and 1-B).

Solanum nodiflorum. In about five days after inoculation light but distinct mottle appears on inoculated leaves. Circular chlorotic spots also develop near or along the veins.

Capsicum annuum. First symptoms of infection in the form of light mottle appear on younger leaves about eight days after inoculation. Older leaves show circular pale spots. Later, the mottling may become more pronounced.

Lycopersicum esculentum. Variety *Sutton's Early Market.* Within 5-6 days after inoculation light green—dark green mottling appears on leaves. The mottle is usually interveinal. There is neither apparent reduction in the size of leaves nor any distortion. About four days later the symptoms become more distinct (Plate IX, fig. 2).

Efforts to transmit the disease to *Petunia hybrida* Vilm. and *Lagenaria vulgaris* Ser., by inoculations with the standard extract were unsuccessful. Immunity of *petunia hybrida* was further confirmed by back inoculation to tobacco.

Reactions on differential hosts of other cultures of *Solanum Virus* I recovered from a large number of plants of *Phulwa variety* showing varying degrees of mottle were also noted. In addition, reactions on differential hosts of several other cultures obtained from plants of *Darjeeling Red Round, Gola* and *Windsor Castle* varieties were studied. Typical symptoms observed are summarized in Table I.

The data given in Table I show that on the basis of reactions on differential hosts the cultures fall into four distinct groups. The first one in which the symptoms produced are in general very faint. This includes culture numbers 1, 2, 5, 6, 7, 8 and 9.

The second group in which the symptoms produced are definite and on some hosts well pronounced. This includes culture numbers 3 and 4.

The third group in which the symptoms produced are highly pronounced and in some cases may even show necrotic lesions. This includes culture numbers 10 and 11.

The fourth group in which the symptoms exhibited on the differential hosts are extremely severe, i.e. resulting in general necrosis and even collapse of leaves (Plate IX, fig. 3). This includes culture number 12.

Properties of the virus

Properties of the culture recovered from plant of *Majestic* Variety and which falls in the third group are described below :

(i) *Thermal-death-point.* Standard extract prepared from young infected tobacco plants was divided into six samples of 3 c.c. each in thin walled glass test tubes of uniform size and capacity. Samples of the standard extract in tubes were exposed to 50°, 60°, 70°, 75°, 80° and 90°C. for ten minutes in a water bath. Every care was taken that the portion of the tube containing the infected plant extract was completely immersed in water. The samples of extract soon after exposure at different temperatures were dipped in cold tap water. Young tobacco plants were inoculated with the samples of standard extract thus exposed and with untreated extract which served as control. The experiment was repeated with the standard extract which had been previously filtered through filter paper. The results of two typical experiments are given in Table II.

FIG. 1. Leaf of infected tobacco var. White Burley, showing circular chlorotic spots

FIG. 2. Leaf of infected *Nicotiana tabacum* showing chlorotic as well as some ring spots

FIG. 3. Leaf of infected *Nicotiana rustica* showing typical concentric spots

FIG. 1–A. Leaf of infected *Datura stramonium* showing early symptoms of infection

FIG. 1–B. Leaf of infected *Datura stramonium* showing intense chlorosis between the veins– A later stage of infection

FIG. 2. Leaf of infected tomato plant var. Sutton's Early Market showing inter-veinal mosaic

FIG. 3. A plant of *Nicotiana tabacum* infected with *Solanum* Virus I (group 4 from potato variety Windsor Castle) showing general necrosis and collapse of leaves

TABLE I

Reactions of certain typical cultures on differential hosts

Culture No.	Source	*Tobacco* var. *White Burley*	*Nicotiana tabacum*	*Nicotiana glutinosa*	*Datura stramonium*	*Capsicum annuum*	*Solanum nodiflorum*	*Petunia hybrida*	*Lycopersicum esculentum* var. *Sutton's early market*
1	Potato var. *Phulwa*	Light mottle with circular chlorotic spots	Light mottle with circular chlorotic spots	Light mottle and circular chlorotic spots	Light but definite mottle tending to disappear after a week	No clear symptoms	No clear symptoms	No symptoms	...
2	*Phulwa* .	Faint mottle and circular chlorotic spots	Faint mottle and circular chlorotic spots	Circular chlorotic spots. A few concentric spots also present	Fleeting mottle .	Faint mottle .	Small circular chlorotic flecks	Do.	Small circular chlorotic flecks on younger leaves
3	*Phulwa* .	Young leaves show distinct circular chlorotic spots which, with an advance in age, develop brown centres. Older leaves show circular chlorotic spots mainly near the veins and a few concentric spots	Distinct circular chlorotic spots on young leaves. Old leaves show mottle or circular chlorotic spots	Circular chlorotic spots on younger leaves along the veins	Light mottle on young leaves	Faint mottle .	Circular chlorotic flecks on young leaves	Do.	Chlorotic flecks on young leaves. Also faint interveinal mosaic
4	*Phulwa* .	Young leaves show very distinct circular chlorotic spots. Older leaves show circular chlorotic or pale spots as well as concentric spots. In a later stage some chlorotic spots develop necrotic centres	Circular chlorotic spots on young leaves. Later faint mottle and concentric spots on older leaves	Circular chlorotic spots on young leaves	Light but distinct mottle	Light mottle on younger leaves	Chlorotic flecks on younger leaves. Older leaves show light mottle	Do.	Distinct mottle and circular chlorotic flecks on young leaves, which later develop interveinal mosaic
5	*Phulwa* .	Young leaves show circular chlorotic spots. Older leaves show light mottle	Younger leaves show circular chlorotic spots. Older leaves show faint mottle and some faint ring spots	Younger leaves show circular chlorotic spots. Older leaves show faint mottle	Light but distinct mottle	Faint mottle	Circular chlorotic flecks on younger leaves	Do.	Mottle and circular chlorotic flecks on young leaves which later develop light interveinal mosaic
6	*Phulwa* .	Circular chlorotic spots on young leaves. Older leaves show faint mottle	Faint circular chlorotic spots on younger leaves	Circular chlorotic spots on younger leaves generally along the veins	Very light mottle .	...	Distinct chlorotic flecks particularly along the veins	Do.	No clear symptoms
7	*Phulwa* .	Circular chlorotic spots on young leaves. Older leaves show light mottle	Circular chlorotic spots on younger leaves	Circular chlorotic spots particularly along the veins	Faint mottle .	Distinct chlorotic spots on young leaves	Faint chlorotic flecks	Do.	Light mottle and chlorotic flecks

TABLE I—*contd.*

Culture No.	Source	*Tobacco* var. *White Burley*	*Nicotiana tabacum*	*Nicotiana glutinosa*	*Datura stramonium*	*Capsicum annuum*	*Solanum nodiflorum*	*Petunia hybrida*	*Lycopersicum esculentum* var. *Sutton's early market*
8	*Darjeeling Red Round*	Small circular chlorotic spots	Faint mottle	Faint chlorotic spots on young and faint mottle on old leaves	Very faint mottle which disappears within five days	A few light green or faint chlorotic areas near the veins	No clear symptoms	Do.	...
9	*Darjeeling Red Round*	Circular chlorotic spots	Light mottle and circular chlorotic spots	Light mottle and circular chlorotic spots	Very faint mottle	No clear symptoms	Faint circular chlorotic spots on some leaves	Do.	...
10	*Majestic*	Very distinct circular chlorotic spots on young leaves. Older leaves develop pronounced cricular pale or chlorotic spots, which later appear as ring spots. Some chlorotic spots may develop necrosis	Symptoms same as in *white Burley*	Very distinct circular chlorotic spots on or along the veins. Some of these spots develop necrosis	Very distinct circular chlorotic spots. Later vein clearing of younger leaves which develop into intense chlorosis between the veins. Some of the chlorotic spots develop necrosis	Distinct chlorotic spots	Distinct circular chlorotic spots	Do.	Distinct interveinal mosaic
11	*Gola*	Distinct circular chlorotic spots some of which later develop necrosis	Distinct circular chlorotic spots some of which later develop necrosis	Distinct circular chlorotic spots some of which later develop necrosis	Distinct circular chlorotic spots. Later young leaves show vein clearing which develops into intense chlorosis between the veins	...	Circular chlorotic flecks particularly along the veins	Do.	Light mottle and chlorotic flecks
12	*Windsor Castle*	.....	Very distinct circular chlorotic spots on all leaves later turning into concentric spots. Finally leaves collapse due to severe necrosis	Distinct circular chlorotic spots. Development of necrosis at a later stage	Chlorotic spots on older leaves. Younger leaves show vein clearing which develops into chlorosis between the veins. Leaves finally develop necrosis	...	First distinct chlorotic flecks and later prominent circular chlorotic spots. Some spots develop necrosis	Do.	Chlorotic flecks, later general, mosaic mottle. Leaves finally develop severe necrotic lesions

TABLE II

Thermal inactivation of the virus

Exposure—Temperture °C.	Experiment I		Experiment II	
	Number of plants		Number of plants	
	Inoculated	Infected	Inoculated	Infected
Unheated control	2	2	3	3
50	3	3	—	—
60	3	3	3	3
65	—	—	3	2
70	3	1	3	1
75	—	—	3	0
80	3	0	3	0
90	3	0	—	—

—denotes not tested

The results given above show that the activity of the virus appreciably falls when exposed to 70°C. and that the virus is completely inactivated at 75°C. or higher. In the plants inoculated with the extract exposed to 75°, 80°, and 90°C., no symptoms of infection were observed even after three weeks.

(*ii*) *Dilution end point.* In order to determine the effect of dilution on infectivity, leaves of young tobacco plants of variety *White Burley* were inoculated with unfiltered freshly extracted pure juice from infected tobacco leaves as well as with juice diluted with sterilized distilled water. Inoculations were also made with standard extract. The experiment was repeated several times and both filtered through filter paper and unfiltered freshly extracted pure juice were tested. The results of one lot of experiments are given in Table III.

TABLE III

Effect of dilution on infectivity

Dilution	Set I Number of plants		Set II Number of plants		Set III Number of plants	
	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected
Nil (pure extract)	4	4	3	3	3	3
Standard extract	3	3	—	—	—	—
1 : 100	3	3	—	—	—	—
1 : 500	3	3	—	—	—	—
1 : 1000	3	3	2	2	3	3
1 : 5000	3	3	—	—	—	—
1 ; 10,000	3	3	2	2	3	2
1 : 12,000	3	3	—	—	—	—
1 : 15,000	3	3	2	2	—	—
1 : 20,000	—	—	3	3	3	1
1 : 30,000	—	—	3	3	—	—
1 : 40,000	—	—	3	3	3	1
1 : 80,000	—	—	—	—	3	1
1 : 100,000	—	—	—	—	3	0

—denotes not tested

The results recorded above indicate that the virus begins to lose its infectivity at a dilution of 1 : 40,000 and is inactivated at 1 : 100,000. In one of the experiments one of the test plants showed faint symptoms of infection even at a dilution of 1 : 100,000, showing that at times it may withstand a dilution up to 1 : 100,000. In dilutions of 1 : 100 to 1 : 10,000 the symptom expression on the test plants was similar and almost of the same intensity as produced by pure and standard extracts.

(*iii*) *Filterability.* Standard extract prepared from young infected tobacco leaves was filtered through filter paper, filter paper impregnated with diatom dust and Pasteur Chamberland filters of grades L_3 and L_5. Prior to passing the standard extract through Chamberland filters it was passed through filter paper impregnated with diatom dust in order to remove as much as possible of the leafy material which tends to clog the filter. All the candles before use were always tested for bubbling pressure and the filtrations were carried out under reduced pressure of 1/5 atmospheric Infectivity of the filtrates was tested on young *White Burley* tobacco plants in the inect proof house. The results of a typcal experiment are given in Table IV.

The results show that the virus passes through all the filters tested and that its activity is not impaired during passage through the filters.

(*iv*) *Longevity in vitro.* Ampoules were filled with L_3 filterate of standard extract which had

TABLE IV

Filterability of the virus

Expt.	Inoculum	Date of inoculation	No. of plants inoculated	Date of infection	No. of plants infected
I	1. Unfiltered standard extract	24-3-43	2	26-3-43	2
	2. Chamberland candle L_3 filtrate	24-3-43	2	26-3-43	2
II	1. Unfiltered standard extract	25-3-43	3	27-3-43	3
	2. Filtered standard extract				
	(*a*) Filter paper (Whatman No. 1) filtrate	25-3-43	3	27-3-43	3
	(*b*) Filter paper impregnated with diatom dust filtrate	25-3-43	3	27-3-43	3
	(*c*) Chamberland candle L_3 filtrate	25-3-43	4	27-3-43	4
	(*d*) Chamberland candle L_5 filtrate	25-3-43	4	27-3-43	4

been freshly prepared from infected tobacco leaves. All precautions were taken to fill the ampoules under aseptic conditions. The ampoules containing the filtered standard extract were stored in a cool cabinet where the temperature varied from 14° to 17°C. and young *White Burley* tobacco plants were inoculated with samples of stored filtrate at various intervals. The fresh filtrate was tested for its infectivity prior to storage.

Results of such an experiment are given in Table V.

The results recorded above show that the activity of the standard extract is not impaired even after being stored for 202 days but the activity falls considerably after storage for 234 days.

TABLE V

Effect of storage of virus

Storage period	No. of plants	
	Inoculated	Infected
Fresh extract	2	2
17 days	3	3
34 days	4	3
46 days	3	2
81 days	3	3
202 days	3	3
234 days	6	1

In another experiment unfiltered standard extract from nfected tobacco plants was stored at room temperature (26·6°-31·1°C.) and it was observed that the extract stored for different periods up to 96 hours proved 100 per cent infectious.

(*v*) *Effect of drying.* 5 c.c. of unfiltered standard extract prepared from infected tobacco leaves was left to dry in a large watch glass at room temperature which varied from 26·6° to 31·1°C. After 10 days when the extract had dried up, 5 c.c. of sterile distilled water was added to make up to the original volume. Three young *White Burley* tobacco plants were inoculated to test the infectivity of the material. Only one of the inoculated plants showed faint symptoms of infection. In another similar experiment filter paper filtrate of standard extract was tested and only three plants out of five showed signs of infection after five days.

The results indicate that the infectivity of the standard extract is greatly impaired but not lost after being dried at room temperature.

(*vi*) *Immunological relationship.* Tobacco plants already infected with a known weak strain of *Solanum virus* I were inoculated with the standard extract obtained from young infected tobacco plants. The second inoculum was introduced 15 days after inoculation with the weak strain in order to give sufficient time for the dissemination of this strain throughout the plant. Also healthy plants were inoculated to serve as check. The plants already infected with a weak strain of *Solanum virus* I continued to exhibit the characteristic faint symptoms of the weak strain but the control plants developed typical pronounced symptoms of infection, showing that the weak strain had immunized the plants against infection by the more virulent strain of the virus.

(*vii*) *Resistance to chemicals.* Effect of absolute alcohol and ether on the standard extract prepared from young infected *White Burley* tobacco plants was determined by exposing 3 c.c. of the

xtract in watch glasses of 2·75 in. diameter to vapours of alcohol and ether in desiccators. The watch glasses containing the extract were removed at regular intervals and the infectivity of the treated extract tested on young tobacco plants. The results of such an experiment are given in Table VI.

The results show that the virus is inactivated after 12 hours' and 32 hours' exposure to vapours of absolute alcohol and ether respectively.

The symptoms on differential hosts and the properties of the virus described show that it is a strain of *Solanum virus* I which resembles, in certain respects, such as dilution end point, strain X^N of Salaman [1938]. The symptoms on the differential hosts are not so pronounced as those described for X^N.

TABLE VI

Effect of alcohol and ether on the activity of the virus

Period of exposure (hours)	Alcohol		Ether	
	No. of plants		No. of plants	
	Inoculated	Infected	Inoculated	Infected
Untreated standard extract	3	3	3	3
4 . . .	3	2	3	3
8 . . .	3	2	3	2
12 . . .	3	0	3	1
24 . . .	3	0	3	2
32 . . .	3	0	3	0

SUMMARY

Properties and reactions on differential hosts of a comparatively virulent strain of *Solanum virus* I isolated from *Majestic* variety *of* potatoes are described.

The virus loses infectivity at a dilution of 1 : 100,000 and exposure to 75°C., for 10 min. is sufficient to destroy the active principle. The virus passes through Chamberland filters of grade L_3 and L_5 without any deleterious effect on its activity. Storage of the virus for 234 days does not render it innocuous.

Reactions on differential hosts of several other cultures of *Solanum virus* I have been studied and the cultures have been divided into four groups on this basis.

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STUDIES ON THE JUTE STEM-WEEVIL *APION CORCHORI* MARSHALL

I. BIONOMICS AND LIFE-HISTORY

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(Received for publication on 28 April 1944)

(With Plate X and four text-figures)

APION corchori Marshall, a stem-borer of jute and commonly known as *jute Apion*, damages the fibre and lowers its quality. Lefroy [1906 ; 1907 and 1909] was the first to record a species of *Apion* breeding in the stem of jute. He regarded it as a minor pest, but visualized the possibility of its causing serious damage. At the Entomological meetings held at Pusa in 1917 and 1919, the consensus of opinion was that it is a minor pest but sporadically serious. In view of this it is not surprizing that the species was not named till in 1936.

Since *Apion* is minute in size and the damage caused by it is not conspicuous, the attention of cultivators has never been focussed on it. In the author's opinion, it is a major pest of jute.

Distribution

The pest is common in Assam, Bihar, Orissa and Bengal. It is wide spread in all the jute-growing districts of Bengal. In Madras, Fletcher [1914] reported it from Bellary and Godavary, and Krishna Ayyar [1940] from Coimbatore.

Nature of damage

The female weevil bores a hole with its rostrum on the stem near the apex of the plant. The egg is generally deposited in between the vascular bundles which are still distinct from one another. In this process the epidermis, cortex, starch sheath and phloem are pierced (Plate X, fig. 4). Very often the cambium itself is injured, and as a result, further development of fibres and wood near the place of injury is checked.

The grub, on hatching, starts feeding on the surrounding tissues and generally tunnels into the pith damaging the fibre. In the pith it may travel up or down for considerable distance, filling the rear of the tunnel with frass. Where the tunnelling is extensive (Plate X, fig. 6) the shoot above withers and numerous side branches are produced. Such plants are undesirable as they yield short fibre. The injury done to seedlings is serious. If they are attacked below the cotyledonary leaves, they die outright. The older plants survive the attack, but remain stunted when attacked repeatedly. Pods are also liable to damage as they are occasionally selected for oviposition.

The place of injury can easily be recognized by the swollen appearance (caused by the abnormal growth of cortical cells, Plate X, fig. 5), and the dark discolouration of the stem or by the withered shoot and axillary bud. Where the epidermis is eaten up, even the excreta may sometimes be seen.

A transverse section passing through the place of injury (Plate X, fig. 5) shows that the mucilaginous substance which oozes out as a result of *Apion* damage firmly binds or cements the adjacent tissues together with the weevils excreta into a hard structure which greatly resists retting. Further, the plant in an attempt to localize injury develops wound cork all around the damaged tissues. This wound cork being suberized also resists retting with the result that at the place of damage the fibre and adjacent tissues are cemented together forming a *knot* (Plate X, figs. 3 and 7). Even after retting, the excreta of *Apion* grub, abnormal cortical cells, epidermis, periderm, fibre bundles and intervening phloem cells are found to form a single compact mass.

Late in the season, ovipositon is generally restricted to the basal part of the stem and the whole bark is found riddled by the grubs, resulting in a hard compact mass with the bark sticking to the fibre. This damage has in the past been described either as specky or knotty, but the term knotty should be restricted to fibre having knots caused by *Apion* injury, while the term specky be used for fibre having fragments of bark adhering to it. A sort of knot may be formed at the nodes due to the leaf and branch traces [Patel, 1943], but the knot caused by *Apion* injury can easily be distinguished by the presence of excreta.

In trade, knots constitute an important defect in fibre quality. These form needles in the sliver and cause breaks in the yarn during spinning, winding and weaving. To the cultivators this defect is known as ' gira ' or ' chouk ', but most of them do not associate it with this tiny weevil.

Life-history of various stages

Pre-oviposition period. As the weevils are almost sexually mature at emergence, the pre-copulation and pre-oviposition periods are rather short. The copulation starts within a day or two after emergence, and is repeated several times a day throughout the season. In ten cultures bred in the laboratory in 1941, the pre-oviposition period varied from 5 to 9 days, the most common being 5 days. In 1942 the pre-oviposition period ranged from 3 to 8 days in fifteen such cultures (Table I). In rare instances, it may extend up to 22 days.

Oviposition. The mode of selection of site for and the process of oviposition are almost similar to those in the cotton-stem weevil, *Pempherulus affinis* [Krishna Ayyar and Margabandhu, 1941]. The female weevil selects for oviposition a place on the stem near the base of petiole and not very far away from the top. Standing on the petiole, it bores a hole with the rostrum, turns back and then deposits the egg in the hole. Only one egg is laid in each hole and the mouth of the hole is sealed with dark excreta. Occasionally more than one hole can be seen in the same neighbourhood but the egg is found only in one hole. Other holes presumably are not suitable for oviposition. Rarely, eggs are laid in the petiole or in the pod. From the middle of July onwards, the eggs are mostly laid in the basal part of the stem. The tops are then rarely attacked. In low-lying areas, where the basal parts of the plants remain under flood water, the weevils have no alternative but to lay eggs in the upper region.

Under laboratory conditions the oviposition period ranged from 6 to 124 days, the maximum

FIG. 1.

FIG. 2.

FIG. 3.

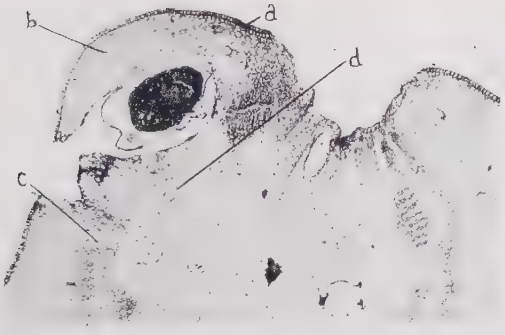


FIG. 4.

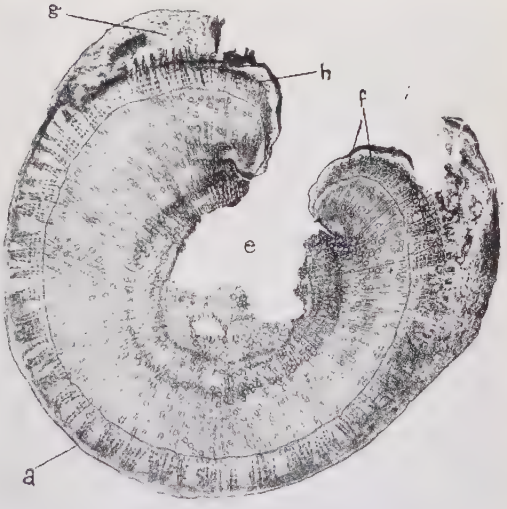


FIG. 5.

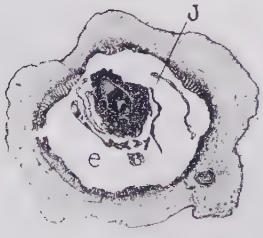


FIG. 6.

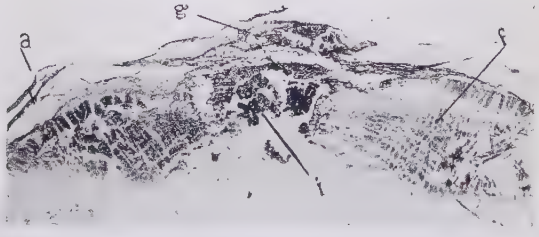


FIG. 7.

TABLE I

Oviposition record

Date of emergence	Date of first oviposition	Number of eggs laid								Total No. of eggs laid	Pre-oviposition period (days)	Oviposition period (days)	Average No. of eggs laid per day
		May	June	July	August	September	October	November	December				
17-7-41	23-7-41	..	..	27	..	..	..	..	..	27	6	6	4·5
18-7-41	Do.	..	..	38	183	89	..	..	..	310	5	61	5·1
Do.	Do.	..	..	67	110	..	..	..	..	177	5	33	5·4
Do.	Do.	..	..	68	58	..	..	..	..	126	5	19	6·6
Do.	Do.	..	..	58	158	136	38	..	..	390	5	81	4·8
Do.	27-7-41	..	..	35	187	18	..	..	..	240	9	40	6·0
16-8-41	22-8-41	..	..	..	61	144	107	14	..	326	6	76	4·3
21-8-41	26-8-41	..	..	..	11	157	101	19	..	288	5	75	3·8
24-8-41	2-9-41	..	..	..	..	119	108	50	17	294	9	101	2·7
3-9-41	10-9-41	..	..	..	..	98	109	12	..	219	7	61	3·6
6-5-42	11-5-42	121	191	127	..	..	..	..	..	439	5	68	6·5
24-5-42	28-5-42	27	178	190	..	..	..	..	..	395	4	65	6·1
Do	1-6-42	..	212	187	116	21	..	..	..	536	8	98	5·8
26-5-42	29-5-42	10	226	201	140	41	..	..	..	618	3	109	5·7
Do	1-6-42	..	196	194	113	8	..	..	..	511	6	94	5·4
26-5-42	Do	..	238	186	71	..	..	..	..	495	6	74	6·7
28-5-42	Do	..	175	183	152	149	16	..	..	675	4	124	5·4
21-6-42	26-6-42	..	28	210	170	134	36	..	..	578	5	106	5·5
23-6-42	Do	..	29	176	127	146	34	..	..	512	3	103	5·0
13-7-42	16-7-42	..	..	90	126	152	10	..	..	378	3	79	4·8
14-7-42	17-7-42	..	..	69	123	127	45	..	..	364	3	69	5·0
16-8-42	19-8-42	..	..	..	76	159	109	..	..	344	3	85	4·1
24-9-42	30-9-42	..	..	..	..	1	117	76	9	203	6	69	2·9
Do	Do	..	..	..	..	1	109	57	4	171	6	65	2·6
15-10-42	20-10-42	..	..	..	..	..	60	69	11	140	5	51	2·7

number of eggs laid being 675 during 124 days. The highest number of eggs laid in a day was 13. The average egg-laying varied from 2·6 to 6·7 per day.

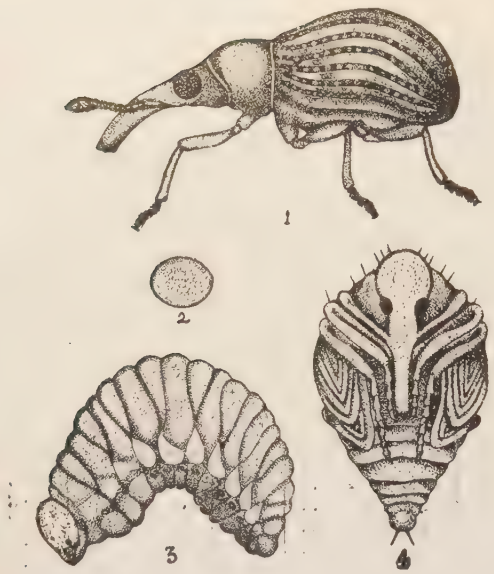
From Table I it is evident that the weevils that emerged in August laid on an average lesser number of eggs than those emerging in July. Irrespective of early or late emergence of the weevil, the egg laying potentiality appears to become reduced towards the end of the jute season, and by December egg-laying practically ceases. The weevils emerging late in season are rarely seen to oviposit soon after.

Incubation period. In the middle of jute season, i.e. in June and July, the incubation within the stem occupies three days at room temperature of 84°-86°F. In a partially dried up stem, it is prolonged to four days. In January when the average mean temperature was 71·9°F., the eggs hatched in five days.

In nature the eggs are deposited in direct contact with the cell sap and are thus protected from desiccation. Freshly laid eggs removed from the stem and left naked in tubes did not hatch either at room temperature or at a constant temperature of 32°C. When, however, the eggs were kept in an atmosphere fully saturated with moisture (with accumulation of fine particles of water in direct contact with eggs), they readily hatched at room temperature or at a constant temperature of 32°C. The eggs could easily be hatched in agar agar media.* Ahmad [1939] observed that the eggs of *Lixus truncatulus* required for their development not only a saturated atmosphere, but perhaps direct contact with free water.

Egg. The egg (Fig. 2) is small, oblong and broadly rounded at both ends. On an average it measures 0·43 mm. in length and 0·33 mm. in breadth. It is white with glistening surface. As it develops it swells up slightly. The brown mandibles of the embryo become visible after two

*2 gm. of agar agar in 100 c.c. of water or jute stem extract



Figs. 1—4. *Apion corchori* Marshall
1. Adult female (×19); 2. Egg (×18);
3. Full grown grub (×16); and 4. Pupa (×18)

days. Before hatching the mandibles are seen to work slowly and the young grub comes out through a slit.

Grub. A freshly hatched grub is slightly longer than the egg. The full-grown grub (Fig. 3) measures on an average 2·85 mm. in length and 0·98 mm. in breadth. The head capsule is small and light brown in colour. The mandibles are dark brown. The body is uniformly light yellow, and well curved, the middle segments being slightly bigger. The terminal segment is broadly rounded at its posterior end.

The mouth-parts of the grub are of usual biting and chewing type, and quite similar to those of *Calandra granaria* [Das, 1939]. The labrum is elliptical with a slight notch at its anterior margin. Each mandible is strongly sclerotized having a broad triangular base, a mesal surface which is produced into two incisor lobes, and a molar or crushing surface near the base. Each maxilla consists of an undivided cardo, a stipes and a two-segmented palpus. The lacinia is present but the galea is totally absent. The labium consists of a membranous postmentum, and a prementum bearing two small two-segmented palpi and a middle lobe.

The grub stage is extremely variable and occupies 8 to 18 days under laboratory conditions. Variation in the larval period might be due to the state of the stem, whether fresh, partially or wholly dry. A similar observation has been made by Krishna Ayyar and Margabandhu [1941] in the cotton-stem weevil, *Pempherulus affinis*, in which a lengthened emergence of weevils is brought about by uneven

Table II

Larval period § at room temperature (78°-90°F.)

Number	Date of hatching	Date of pupation	Larval period (days)
1	8 June .	20 June .	12
2	17 June .	25 June .	8
3	20 June .	29 June .	9
4	23 June .	6 July .	13
5	25 June .	9 July .	14
6	29 June .	11 July .	12
7	3 July .	14 July .	11
8	5 July .	15 Juy .	10
9	6 July .	24 July .	18
10	8 July .	23 July .	15

§ Only a cross-section of the entire data

development of the mature grubs, owing to either insufficient nutrition or extreme dryness or dampness of the stem.

Pupa. When full-fed, the grub makes a rough chamber of excreta inside the stem and pupates within it. Before pupation the abdomen of the grub becomes straight and the prepupa looks longer than the grub. The pupa (Fig. 4) measures about 2·07 mm. in length and 1·08 mm. at its maximum width. The body is pale yellow gradually turning light brown. The eyes are reddish brown in the beginning but afterwards they turn black. The wing pads lie between the second and third pairs of legs, and extend beyond the abdomen. The abdomen is slightly curved. The rostrum lies ventrally. The legs are folded and the antennæ run parallel to the tibiæ.

The duration of the pupal period inside the stem at room temperature of 84°-85°F. is about four days, but when the pupa is left naked, the pupal period is slightly shortened. The adult weevil emerges through the passage previously made by the grub during feeding, or by making a fresh hole.

The whole life cycle from egg up to emergence of the adult is completed in 15 to 25 days.

Adult. It is a small tiny weevil (Fig. 1), about 1·8 mm. in length and 0·8 mm. in breadth, with the curved snout very conspicuous. It is dark brown or dull black clothed with sparse whitish setæ almost throughout the body. The female is slightly bigger than the male. The detailed description of the weevil has been given by Marshall [1936].

Longevity. The life of the weevil is fairly long. The maximum age recorded under captivity was 209 days and the minimum 11 days. The average for 153 weevils was 81 days (Table III).

TABLE III

Longevity of weevils

Number of weevils observed	Maximum life (days)	Average life (days)	Weevils emerged on
10	121	65·9	17-6-41
10	108	53·4	28-6-41
12	190	106·3	16-7-41
13	209	120·2	16-7-41
10	162	62·9	19-9-41
10	119	75·9	4-11-41
15	119	97·4	15-11-41
10	98	66·8	1-6-42
10	112	66·2	14-6-42
10	117	65·1	15-7-42
10	121	69·7	21-9-42
12	153	97·2	24-9-42
10	116	102·9	2-10-42
11	127	63·8	1-11-42
			Old weevils collected on
10	76	45·2	2-5-41
10	75	45·5	3-5-41
16	93	71·1	1-5-42
20	123	78·3	1-5-42

NUMBER OF BROODS AND SEASONAL HISTORY

There is much overlapping of the rapidly succeeding broods and the crop is heavily infested with all stages. The weevils emerge throughout the crop season without any break. The overwintering weevils breed for about two months and a half. Excepting those emerging in November all weevils breed in the same season, but the breeding activity declines as the jute season advances.

The weevils can be seen on the crop throughout the jute season, and their presence can be detected by small pin-holes made on tender leaves during feeding. They drop on the ground at slight disturbance and feign death. The weevils have been observed to attack self-sown plants as early as in January. In low lying areas where self-sowing does not occur, the weevils have been seen on very young seedlings about 2 in. in height by the middle of March.

As immediately after the harvest of jute the immature stages are found in the stubbles, it was thought that either the grubs or pupæ might pass the winter in hibernation in the stubbles, or in soil around the stubbles. Examination of stubbles and surrounding soil carried out during the winter failed to reveal the presence of any immature stages of the pest. Under laboratory conditions at a temperature of 71°-78°F. and mean relative humidity of 86 per cent the grubs in the stubbles did not enter the soil for pupation, but pupated in the stubbles and emerged as weevils, during the later part of November ; neither the larval period nor the pupal period was prolonged.

The adult weevils pass the winter in concealment in bushes, shrubs and hedges. *Streblus asper* Lour, *Glycosmis pentaphylla* Corr., var. *nitida*, *Ziziphus eonoplia* Mill, *Blumea lacera* D.C., *Pithecolobium dulce* Benth, *Hibiscus rosa-sinensis* Linn., *Gossypium spp.*, *Duranta plumieri*, and Guinea grass (*Panicum maximum* Jacq.) are seen to afford shelter to the weevils during the off season, but the insect was never found to breed on them. On any of these plants, the weevils did not feed in the laboratory. When supplied with water the weevils lived without food up to a maximum of 35 days. It may be that under natural conditions they can live for a longer period without food and these conditions could not be reproduced in the laboratory.

Alternate host. Intensive search has so far revealed only one alternate host, viz. *Triumfetta rhomboidea* Jacy, locally known as *bun-okra*, a fibrous weed belonging to the family *Tiliacae*. It is widely distributed throughout tropical and, sub-tropical regions of India and Ceylon, ascending up to 4,000 ft. in the Himalayas.

The weevils feed on the leaves of this plant and occasionally breed in the stem. In winter the grubs and pupæ have been collected from the infested stems of *bun-okra*. In feeding experiments with jute and *bun-okra* leaves, the weevils did not show any preference. During the season *bun-okra* plants growing near the jute fields are found less infested than jute. The stems of *bun-okra* are not so soft and succulent and may not be so suitable as those of jute for the development of the grubs. Krishna Ayyar [1940] reports *Triumfetta rhomboidea* as an alternate host of the cotton-stem weevil (*Pempherulus affinis* Fst.) which also occasionally breeds in the stem of jute (*C. olitorius*).

VARIETAL SUSCEPTIBILITY

Five species of *Corchorus* have been under observation for three seasons. *C. acutangulus* in spite of the presence of long hairs on the leaf, petiole and stem is highly susceptible to *Apion* attack. *C. trilocularis* and *C. fascicularis* are less susceptible than *C. acutangulus*. All the above three are wild species of jute.

Among the two cultivated species, *C. olitorius* is less susceptible than *C. capsularis*. The following records taken from botanical trials conducted in the same field show that *capsularis* varieties are in general more susceptible than *olitorius* ones, though in certain years the difference between the two species may become negligible.

With a view to assessing varietal resistance a trial of 49 types was carried out in the years 1939, 1940 and 1942. The variation in susceptibility to *Apion* attack was found to be limited in the case of *olitorius* varieties, whereas the *capsularis*

TABLE IV

Infested plants per cent

	1939	1940	1942
Olitorius varieties			
R. 26 . . .	13·8	65·3	93·3
C. G. . . .	17·2	59·3	98·7
Dacca local . .	23·1	61·0	99·0
Critical difference .	7·2	Insignificant	3·4
Capsularis varieties			
D. 154 . . .	57·4	82·1	98·7
Fanduk . .	59·7	78·2	95·7
D. 386 . . .	65·2	78·7	98.0
Critical difference .	Insignificant	Insignificant	2·2

varieties exhibited rather a wider range. The resistance of *olitorius* may possibly be due to its higher tannin content, which is about three times more than in *capsularis* stems. In feeding experiments the weevils preferred *capsularis* leaves (which are found to contain less tannin) to *olitorius* ones.

EXTENT AND INTENSITY OF INFESTATION

Assessment of damage. In gauging the damage, the percentage of infested plants as well as the number of punctures in such plants are important. From Table V it is evident that about 50 per cent of *capsularis* plants were attacked three times or more, whereas in *olitorius* only 17 per cent of the plants had three or more punctures. In general, the greater the number of plants infested, the higher was the intensity of attack. As many as 33 punctures were recorded on a *capsularis* plant in a pot culture. Lefroy [1907] records that in some cases one grub is found to almost every leaf.

TABLE V

Extent and itensity of Apion *infestation*

Number of punctures per plant	Frequency of infested plants (mean per cent)	
	Olitorius varieties C. G., R. 26 and Local	*Capsularis* varieties D. 154, D. 386 and Fanduk
0	38·58	16·63
1	25·09	16·29
2	19·22	17·19
3	10·74	19·80
4	4·37	14·82
5	1·37	7·92
6	0·25	3·72
7	0·12	2·04
8	0·12	0·68
9	0·00	0·57
10	0·00	0·23
11	0·00	0·00
12	0·00	0·00
13	0·00	0·00
14	0·00	0·11
15	0·00	0·11

Thirteen commercial samples obtained from the Director, Technological Research Laboratories, Calcutta, were examined for *Apion* damage. In each sample 'reeds' of 100 plants were examined. The percentage of damaged 'reeds' varied from 9 to 91 and the intensity of attack from 1·00 to 4·14 per attacked 'reed'. The amount of damage may best be expressed as an average number of *Apion* punctures per plant, irrespective of the plant being healthy or attacked.

TABLE VI

Damage in commercial fibre

Sample No. of T. R. L.	Attacked 'reeds' per cent (extent)	Average No. of knots in attacked 'reeds' (intensity)	Amount of damage (extent × intensity) 100
J. 93 . .	9	1·11	0·10
J. 87 . .	11	1·18	0·13
J. 97 . .	13	1·00	0·13
J. 91 . .	14	1·50	0·21
J. 86 . .	36	1·77	0·64
J. 92 . .	41	1·09	0·78
J. 94 . .	50	1·64	0·84
J. 90 . .	54	1·48	0·80
J. 89 . .	60	1·51	0·91
J. 95 . .	64	1·73	1·09
J. 96 . .	65	1·94	1·26
J. 85 . .	67	2·04	1·37
J. 88 . .	91	4·14	3·77

With a view to note the amount of damage at different places, fibres obtained from quality trials were examined. From the results furnished in Table VII, it may be seen that in *capsularis* the Rangpur fibre recorded the maximum damage and the Faridpur one the least. The *olitorius* fibre from Berhampore, Chinsurah and Pabna recorded less knots than that of Dacca. *Apion* attack is wide-spread in Bengal and variation in damage from place to place is to be expected. Whether the incidence of *Apion* is less in pure *olitorious* tracts such as Chinsurah, Berhampore and Pabna is a point which requires further investigation and survey. Since the weevil prefers *capsularis*, it is to be expected that its incidence is more where only *capsularis* or both *capsularis* and *olitorius* are grown as at Dacca.

That the knots do not result from all the punctures is evident from the fact that the number of punctures observed in the standing crop is consistently higher than the number of knots in the corresponding fibre. It is, however, clear that a fair estimate of damage can be obtained even from the examination of fibre samples.

The crop around the borders is subject to heavier attack than the inner one. It may be seen from Table VIII that the percentage of infestation as well as its intensity are higher in the border crop.

TABLE VII

Amount of damage in quality trials, 1940

	Average No. of knots per 'reed'			Mean average knots in three varieties
	C. G.	R. 26.	Local	
Olitorius varieties	..	..	..	..
Pabna fibre	0·20	0·27	0·19	0·22
Berhampore fibre	0·08	0·11	0·07	0·08
Chinsurah fibre	0·13	0·11	0·12	0·12
Dacca fibre	0·66	1·00	0·38	0·68
Standing crop, Dacca	1·09	1·45	1·19	1·24
Capsularis varieties	D 154	D 386	Fanduk	..
Rangpur fibre	5·13	4·48	4·51	4·70
Faridpur fibre	1·55	1·12	0·90	1·19
Dacca fibre	1·53	1·77	1·59	1·63
Standing crop, Dacca	2·45	2·54	2·57	2·52

TABLE VIII

Direction of attack

	Capsularis border	*Capsularis* centre	*Olitorius* border	*Olitorius* centre
Per cent infested plants	97·77	86·95	73·22	59·71
Average number of punctures in infested plants	8·11	3·44	2·44	2·04
Average number of punctures in total plants	7·93	2·98	1·79	1·22

After the harvest of jute in low-lying areas such as *char* and *beel-lands*, the weevils fly to high-lands near the villages. They pass the winter in shrubs, weeds, bushes and hedges, and as soon as the new crop is available they come out and attack the nearest fields. Further away from the winter shelter, the attack, therefore, becomes gradually lighter.

Soil fertility. To find out the influence of fertilizers on *Apion* infestation, three years data from a manurial experiment conducted on the Dacca Farm were statistically analysed. It was found that the plots manured with super-phosphate in combination with calcium, or with ammonium sulphate in combination with super-phosphate and muriate of potash or potash alone suffered significantly less *Apion* infestation than the control farmyard manure plot. In general, nitrogenous manures increase *Apion* infestation, but potash and phosphatic fertilizers decrease it.

TABLE IX

Effect of fertilizers on Apion *infestation*

Treatments	Years			*Apion* infestation Average mean per cent
	1939	1940	1941	
fym	416·5	286·8	280·4	54·6
n	446·7	315·6	267·4	57·2
p	363·5	279·5	289·3	51·7
np	312·1	356·4	246·6	50·8
k	400·4	224·5	186·8	45·0
nk	343·6	377·7	307·7	57·1
pk	355·7	250·7	214·0	45·5
npk	311·5	246·7	205·5	42·4
ca	386·1	278·6	293·1	53·2
nca	445·2	299·8	304·8	58·3
p ca	324·5	234·7	170·3	40·5
np ca	362·8	319·0	231·1	50·6
k ca	427·7	208·1	267·8	50·2
nk ca	273·9	265·5	274·7	45·2
pk ca	367·8	259·5	208·1	46·4
npk ca	316·9	249·1	311·0	48·7
Critical difference	Insignificant	87·7	Insignificant	7·7

n = ammonium sulphate at 488 lb. per acre
o = super-phosphate at 314 lb. per acre
k = muriate of potash at 150 lb. per acre
ca = lime at 10 md. per acre
fym = farmyard manure (basal dressing for all treatments) at 100 md. per acre

Influence of date of sowing. Apion infestation of the top shoot is comparatively high in the early-sown crop than in the late-sown one, as from July onwards the weevils prefer laying eggs in the basa part of the stem. The late-sown crop is, therefore, attacked less at the top and more at the base. In a *capsularis* crop sown in the middle of July at two and half months' age, tops and stem of only 5 per cent plants were infested, but the basal region of the stem of almost every plant was attacked, and immature stages were present therein.

Damage and disease. Apion punctures, particularly at the basal region, facilitate the entry of fungi like *Macrophomina Phaseoli* and *Sclerotium rolfsii*.

PARASITES

Though the pre-imaginal stages are passed inside the stem protected from the enemies, a high percentage of the grubs are subject to attack of two parasites. They await identification with the Imperial Entomologist, Imperial Agricultural Research Institute, New Delhi. One of the parasites belongs to the family *Miscogastridae* (*Chalcidoidea*) and could not be referred to any genus hitherto described. The other belongs to the family *Braconidae* (*Ichneumonoidea*).

The female of both the parasites is able to locate the grub that has not already been parasitized and paralyzes it by injecting poisonous fluid. The grub becomes inactive and the egg is deposited on its body. The parasitized grub from which a freshly laid egg or newly hatched larva (of parasite) has been removed does not remain alive for a long time. In no case more than one egg was found to have been deposited on the body of a single host. So far only one pupa parasitized by the *Chalcidoid* has been recorded. Soon after hatching, the braconid larva fixes itself to the body of the host for imbibing body fluid. The *Chalcidoid* larva enters the body of the host and feeds within. The full-fed larva pupates inside the stem.

The parasites are very useful in controlling the pest as they cause over 50 per cent mortality of the grubs (Table X). In the beginning of the jute season the parasitization is low, it reaches an optimum level in the mid-season, and declines towards the end.

TABLE X

Incidence of parasites

Months	Number of grubs examined	Per cent parasitized grubs	Collected from
1939	190	62·6	Top and mid-stem
1940—			
June	447	36·9	Do.
July	419	57·5	Do.
August	121	57·2	Do.
September	469	55·1	Do.
Do.	88	14·8	Stubble
October	35	17·1	Do.
Do.	32	9·4	Self-sown plant
1941—			
May	64	17·6	Top and mid-stem
June	157	28·7	Do.
July	142	57·0	Do.
August	222	78·8	Do.
September	20	85·0	Top of stem only
Do.	135	35·8	Stubble
October	184	37·0	Do.
Do.	191	28·8	Basal part of stem
November	539	48·9	Stubble
1942—			
May	113	29·2	Top and mid-stem
June	215	28·8	Do.
July	308	65·6	Do.
August	206	84·5	Do.
September	19	89·5	Top of stem only
Do.	55	49·1	Stubble
Do.	1948	64·4	Basal part of the stem
October	2056	45·4	Do.
Do.	148	62·8	Entire plant
Do.	339	48·1	Stubble

CONTROL MEASURES

The insect completes its life-history in a protected state inside the stem and thus presents a most difficult problem. The adults are very small, and though found on the crop throughout the season, take very little food and as such cannot effectively be dealt with by any insecticide.

Lefroy [1907] states that unfortunately there does not appear to be any practical method of dealing with such a small insect, but suggests that it may be possible to get a trap crop for it. So far, no alternate host other than *bun-okra* (*Triumfetta rhomboidea*) has been found. As the weevil prefers to breed on jute, it is not possible to use *bun-okra* as a trap crop.

Over 50 per cent of the pest is destroyed by the two parasites. The importance of these parasites in checking the pest is very high. Pending the investigation of the question of utilizing the parasites for controlling the pest, the following control measures are suggested.

1. Destruction of immature stages by burning or retting of the infested plants during the early part of crop season, so that the activities of the pest can be checked right in the beginning. Infested plants can easily be located from their withered tops. The removal of infested plants during the early part of crop season will not affect the yield, as the crop has to be thinned subsequently. The plants removed during thinning should be collected and destroyed by submerging them in water.

2. Destruction of immature stages in the stubbles by collecting and burning or submerging stubbles immediately after the harvest of jute. The fields should be ploughed soon after harvest.

3. The self-sown plants which are found to be infested with the pest should be destroyed by ploughing or harrowing.

4. Destruction of immature stages by quick steeping of harvested jute. For easy handling and retting it is customary to stack the harvested jute for some time before it is steeped. For the first five days more parasites emerge from the cut jute than the weevils, but subsequently more weevils emerge than the parasites. It is, therefore, desirable not to stack the harvested jute for more than four days.

SUMMARY

Apion corchori Marshall, commonly known as the jute *Apion*, is a very important pest of jute affecting the quality of fibre. It is widely distributed in Bengal and other jute-growing provinces.

The eggs are laid singly in the stem near the base of the petiole, not very far from the apex. Occasionally the petiole and seed pods are selected for oviposition. During the late season oviposition is mostly confined to the basal part of the stem.

The maximum number of eggs laid by a female during an oviposition period of 124 days was 675. The highest number of eggs laid in a day was 13. The incubation period at room temperature is

3 days during summer and 5 days in January. The grub stage is extremely variable and occupied 8 to 18 days according to nutrition available in the stem. The pupal period is four days.

The maximum life of a weevil in captivity was 209 days. During the winter, a weevil lived for 35 days on water without any food.

Throughout the season, due to continuous multiplication by successive generations, the crop is heavily infested with all stages.

The adult causes very little damage. It is only the grub that is responsible for the damage which results in the formation of knot in the fibre deteriorating its quality.

Of the two cultivated species the *olitorius* is less susceptible. This might be due to higher tannin content in the latter's stem.

In gauging the damage, the percentage of infested plants as well as the intensity of infestation (number of punctures per plant) should be taken into consideration.

The crop around the borders is subject to heavier attack than the inner one. Away from the weevil's winter shelter the attack becomes gradually lighter.

The early-sown crop is attacked mostly at the top, and the late-sown at the base of the stem.

The plot manured with super-phosphates in combination with calcium or with ammonium sulphate in combination with super-phosphate and potash is less attacked by *Apion*.

In general, application of phosphatic and potassic fertilizer decreases *Apion* infestation, whereas the nitrogenous manures tend to aid its attack.

Two parasites are found to check the pest. They destroy over 50 per cent of the grubs.

The control measures suggested are based on the destruction of immature stages of the pest early and late in the season by destroying the infested material.

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EXPLANATION OF PLATE X

Fig. 1. Infested jute stem ; a—injury

Fig. 2. Split jute-stem showing the grub about to pupate ; b—grub

Fig. 3. Knotty fibre

Fig. 4. Transverse section of stem showing an egg ; a—epidermis ; b—cortex ; c—phlœm ; d—abnormal tissue growth (×40)

Fig. 5. Transverse section of stem at the place of injury ; a—epidermis ; e—tunnel ; f—deformed fibre bundles ; g—abnormal cortical cell-growth ; h—periderm (×8)

Fig. 6. Transverse section of stem showing tunnel made by the grub ; e—tunnel ; j—pupa (×12)

Fig. 7. Transverse section of knot ; a—epidermis ; f—fibre bundles ; g—abnormal cortical cells ; i—excreta (×12)

BIOLOGY AND CONTROL OF MAIZE AND *JOWAR* BORER (*CHILO ZONELLUS* SWIN.)

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MAIZE and *jowar* are amongst our most important grain crops. Though both are grown all over India, maize is more extensively cultivated in Bihar and North West Frontier Province, and *jowar* in Bombay, Central Provinces, Berar, Madras and Sind, while in the Punjab and the United Provinces they occupy extensive and almost equal acreage.

These crops are attacked by a number of insect pests of which maize and *jowar* borer (*Chilo zonellus* Swin.) is the most destructive, particularly in the Punjab where it has been under investigation for a number of years. This paper embodies the results of these investigations.

LIFE HISTORY*

Habits and response to light. Moths are nocturnal. During day they were found to rest on

*Most of the information given under this heading is taken from the annual reports of Bh. Amrik Singh (now working as Plant Protection Assistant at Hansi) who worked on this pest from February 1934 to October 1937 under the supervision and guidance of my predecessors, M. Afzal Husain and (the late) Dr R. L. Chopra, and myself

tree trunks, under clods and stones and among leaves. They are positively phototropic ; during July-September their response to light was found to be at its height between 8 and 10 P.M.

Longevity of adults. The moths are short-lived ; male and females observed from emergence to death lived respectively for 2-6 and 2-7 days in April ; 1-4 and 2-6 days in May ; 1-2 and 1-3 days in June ; 1-3 and 1-3 days in July and August ; and 1-2 and 1-2 days in September.

Number of eggs in a cluster and its size. Females lay oval, dull creamy eggs (which become black before hatching) in clusters on leaves. Each cluster contained 10-36, with an average of 23 eggs and measured from 3·1-12·0, with an average of 6·2 mm. in length and from 1·0-3·0, with an average of 20 mm. in breadth.

Progress of oviposition in the fields. The moths from the over-wintered larvae started oviposition in the fields in the last week of March, the subsequent generations of moths maintaining it right up to the end of October. Thus the egg clusters of *C. zonellus* were met with in the fields from the end of March to the end of October. These egg clusters were regularly collected from different fields in order to study the progress of oviposition. The results of these observations are given in Table I.

TABLE I

Progress of oviposition by Chilo zonellus *in the fields during March-October*

Month	Total number of plants examined in different fields	Number of egg-clusters found on these plants	Average number of eggs laid per month per 1000 plants
March (4th week)	1,000	3	3·0
April	6,000	149	24·8
May	10,500	30	2·8
June	9,500	22	2·3
July	17,000	118	6·9
August	14,100	256	18·1
September	25,300	77	3·8
October	22,500	46	2·04

It will be observed from the above table that the pest laid the largest number of eggs in April and August and fewest number of eggs in May, June and October.

Location of egg-clusters. According to Kuwayama [1928] *C. zonellus* lays its egg-clusters on the distal portion of a leaf. In the Punjab the position of 150 egg-clusters was studied on the leaves during April-June and it was found that of the total number of egg clusters laid on a leaf, 5·3 per cent of them were laid on the distal half, and the remaining 94·7 per cent within 2-5 cm. of the base of leaf.

Egg-stage. G. E. [1918], Ishikawa [1922] and Kuwana [1929] have found the egg-stage to occupy a week, 8-9 days and a week in Mesopotamia, Niigata and Japan respectively. In the Punjab duration of the egg-stage was studied for 4 years and it was found that the eggs laid in April hatched in 2 days while those laid during May-September and October took 3-4 and 5 days respectively to hatch (Table II).

Larval stage. G. E. [1918], Ishikawa [1922] and Kuwana [1929] have found the larval stage to occupy 30, 40-50 and 24-56 days in Mesopotamia, Niigata and Japan respectively during the active period. Duration of the larval stage of this pest was studied in the Punjab for 3 years and it was found that during the active period (i.e. April-September) it occupied 15-31 days (Table II).

Pupal stage. Kuwana [1929] has found the pupal stage to be completed in 7-8 days in Japan during the active period. With us in the Punjab the pupal stage had been found to occupy 2-9 days during April-September (Table II).

Duration of the pupal stage of the over-wintered larvae has been studied by Kuwana [1929] and Katsumata [1934] who found it to occupy 15 and 22·4 days respectively in Japan. In the Punjab pupal stage of the over-wintered larvae had been found to last for 8-15 days in March-April, and 7-8 days during May.

Duration of life-cycle. The pest completed its life-cycle in 24-39 days (Table II)

Number of generations. According to Kuwana [1929] *Chilo simplex* (*zonellus*) Butl. has two generations a year in Korea and most of Japan, one in the north and four in Formosa while according to Pang Hwa Tsai [1932] it has two, occasionally three, generations a year in China and according to Ishikawa [1922] it has two generations a year in Niigata. Number of generations of this pest was also studied in the Punjab. For this purpose it was reared continuously in muslin cloth sleeves on maize plants and it was found that it had 6-7 generations a year which overlapped each other.

Location of hibernating caterpillers. C. zonellus hibernates as a caterpillar from the 2nd week of October to the end of March. Observations made to study the location of hibernating caterpillars in the maize plant when the crop was being harvested during November-December. showed that 76·7 per cent of the caterpillars hibernated in stubbles, 13·5 per cent in stems and 9·8 per cent in cobs.

Almost all the caterpillars hibernating in stems and cobs are killed during winter as the former

TABLE II

Duration of life-cycle of C. zonellus

Eggs laid on	Eggs hatched on	Larvae pupated on	Adults emerged on	Duration of (in days)			Duration of life-cycle (in days)
				Egg stage	Larval stage	Pupal stage	
4 April . .	6 April . .	27 April—7 May .	6 May—13 May .	2	21—31	6—9	32—39
12 May . .	16 May . .	3 June—11 June	10 June—15 June	4	18—26	4—7	29—34
13 June . .	16 June . .	4 July—7 July .	11 July—14 July	3	18—21	7	21—31
13 July . .	17 July . .	1 August 13 – August	10 Aug.—18 Aug.	4	15—27	5—9	28—36
11 August .	15 August . .	2 September—12 September	8 September—14 September	4	18—28	2—6	28—34
12 September .	15 September .	30 September—5 October	6 October—13 October	3	15—20	6—8	24—31

are used as dry fodder for cattle and the grain from the latter as feed for man and livestock.

Similar observations were also made in connection with *jowar* and it was found that 12·2 per cent of the caterpillars hibernated in stubbles and 87·8 per cent in stems: in the stems they were mostly (79·0 per cent) found from 6-72 in. from the ground.

Emergence of moths from hibernating caterpillars. Emergence of moths from hibernating caterpillars was studied for three years. For this purpose a large number of caterpillars hibernating in stubbles and cobs of maize and *jowar* were kept in the laboratory. From stubbles, the emergence always started in the 1st week of April and was continued up to the 3rd week of June; 90·0 per cent of the moths emerging between the 2nd week of April and the 2nd week of May. From the cobs the emergence always started in the 3rd week of April and continued up to the end of June, 65·0 per cent of the moths emerging in May.

DAMAGE

C. zonellus does the most damage to grain rather than fodder crops of maize and *jowar*. It attacks all parts of the maize plant except roots; it bores in its stem and feeds on its leaves and cobs. In *jowar* it bores in the stem which is ridded with holes. Attacked plants often bend or twist over, and produce branches. Such plants remain stunted and die without producing grains. The attacked plants show 'dead-hearts', the bases of which smell offensively.

The caterpillars also bore into cobs and feed on ripening grains. 5445 cobs were examined at the time of harvest on 17, 27 and 29 November; 3, 8, 9, 11 and 12 December; and 20 January and the percentage of attack on them was found to vary from 2·6 to 24·3. Attacked cobs are further spoiled by the caterpillars voiding their whitish excreta on them.

RESISTANCE OF LOCAL AND FOREIGN VARIETIES OF MAIZE TO *C. Zonellus*

Punjab selected varieties. Experiments on the comparative varietal susceptibility of red Nos. 1, 2, 3, white No. 1 and local variety of maize to the maize and *jowar* borer were carried out and the percentage of attack on these varieties as recorded two months after sowing was found to be 20·1, 30·0, 20·7, 29·0 and 18·2 respectively.

U. S. A. varieties. Michigan corn hybrid 561 is claimed to be resistant to the European corn borer (*Pyrausta nubilalis* Hb.) in Michigan, U. S. A. To test its resistance to *C. zonellus*, 5 lb. of its seed was obtained through the courtesy of Mr A. R. Marston, Michigan State College of Agriculture and Applied Science, East Lansing, U. S. A., and sown at Lyallpur along with the local variety of maize. The percentage of attack was calculated about a month after sowing. The results of the two consecutive years (1939 and 1940) showed that Michigan corn hybrid 561 was as severely attacked and damaged by *C. zonellus* as the local variety of maize. Therefore, because of this fact and because of its poor yield and poor stand as compared to the local variety, Michigan corn hybrid 561 was discarded and the following other American corn hybrids (obtained through the courtesy of Mr Merle T. Jenkins, Principal Agronomist-in-Charge of Corn Investigations, United State Department of Agriculture, Bureau of Plant Industry, Washington) were tried at Lyallpur along with the local varieties during 1942. The results of these trials are given in Table III.

TABLE III

Resistance of American and local varieties to Chilo zonellus

(Date of sowing 10 August 1942)

Variety	Number of plants examined on 26 November 1942	Number of caterpillars found	Yield of grain per acre
			Md. sr. ch.
American—			
$B^2 \times$ Pr.	200	20	27 2 0
$I11 \times 960$	200	5	38 2 0
$B^2 \times$ 38-II	200	9	39 35 0
$R^2 \times$ L317	200	13	11 0 0
$R^4 \times$ HY	200	8	13 3 0
$B^2 \times$ WF9	200	8	24 3 0
Local—			
Local (indigenous)	200	13	37 27 0
M. S. 28	200	16	33 19 0
U. S. 44	200	15	37 8 0
M. S. 63	200	10	27 13 0

The above table gives the results of the first year of the American varieties of corn under Punjab conditions. These trials are being continued and their results will be communicated in due course.

EFFECT OF STIMULATING PLANT GROWTH ON *C. Zonellus* INCIDENCE

When doing field work on *C. zonellus* it was noticed that when a maize plant was attacked before nodes were formed it showed a 'dead heart' and its growth was checked, but when it was attacked after node formation, it neither produced a 'dead heart' nor its growth was checked. It was, therefore, decided to study the effect of stimulating plant growth for earlier node formation by the application of nitrogenous manures on the incidence of pest. For this purpose experiments were laid out in 1940 and again in 1942. Since the results obtained in 1942 confirm the data obtained in 1940, findings of only 1942 are discussed.

In 1942 an area of 118 × 66 ft. was selected and divided into four blocks, each block being further sub-divided into four plots. The following four treatments with four replications of each were given by block randomization system: (1) Control, (2) Farm-yard-manure at the rate of 50 cart loads per acre, (3) Ammonium sulphate at the rate of 4 md. per acre added about a fortnight after sowing, (4) Farm-yard-manure and ammonium sulphate at the above rates. Maize was sown on 30 July. The plots were irrigated immediately after the addition of ammonium sulphate.

The damage was recorded in the first week of October. It varied from 16·0 to 18·25 per cent in the treated and control plots as is seen from Table IV: the results obtained were negative.

TABLE IV

Incidence of the pest in the control and treated plots

Treatment	Number of plants		Percentage of attack
	Examined	Attacked	
Control	400	71	17·7
Farm-yard-manure	400	73	18·25
Ammonium sulphate	455	72	16·0
Farm-yard-manure plus ammonium sulphate	400	71	17·7

MORTALITY AMONGST CATERPILLARS HIBERNATING IN MAIZE STUBBLES IN BERSEEM FIELDS

As pointed out above maize in the Punjab is generally followed by such fodder crops as 'senji' (*Melilotus parviflora*) or 'berseem' (*Trifolium-alexandrinum*) but 'berseem' is now more popular with the cultivators and is more extensively grown. Maize stubbles from 'berseem' fields were examined for hibernating borers. The results are presented in Table V.

TABLE V

Mortality percentage among caterpillars hibernating in maize stubbles in berseem fields

Month and week	No. of stubbles examined	No. of larvae found		Mortality percentage
		Living	Dead	
March				
I week	200	46	1	2·2
II week	300	63	2	3·0
III week	400	22	7	24·1
IV week	500	60	24	28·5
April				
I week	300	23	9	28·1
II week	300	28	17	37·7
III week	300	22	18	45·0
IV week	400	28	8	22·2
May				
I week	400	22	9	29·0
II week	200	9	5	35·7
III week	400	10	10	50·0

It will be observed from the above table that mortality amongst the caterpillars hibernating in maize stubbles in 'berseem' fields is quite appreciable and during 3rd week of March to 3rd week of May it ranged from 22·2 to 50·0 per cent.

The effect of date of sowing on the incidence of *C. Zonellus*

Experiments to study the comparative borer attack on maize sown on different dates were started in July 1935 and concluded in August 1942. These experiments were laid out at Montgomery, Lyallpur, Beas (Amritsar district) and Rawalpindi. Maize is an important crop and maize borer a serious pest in these localities. The experimental field selected for the purpose at all these places was divided into four blocks, each block being further sub-divided into four plots. Maize was sown four times during the season nearly after fortnightly intervals starting from mid-July and the sowings were done by block randomization system. Thus in all cases there were four sowings with four replications of each. The area of each experimental plot was 1/40th of an acre. Non-experimental strips were provided at each end of these plots.

In order to record the incidence of the pest, maize was examined 1-2 months after sowing. For this examination, three equal patches were selected at random from each plot and all the plants in them cut close to the ground and examined for caterpillars.

A large mass of experimental data have thus been collected and as all these data lead to, and confirm, the same conclusions only one year's results are given for each locality for the sake of brevity.

Table VI

Comparative C. zonellus *attack on maize sown on different dates*

Locality and year	Date of sowing	Date of examination	No. of plants		Percentage of attack	Population of borers per 100 plants
			Examined	Attacked		
Lyallpur, 1939	18 July	18 August	314	292	93·2	366·5
	31 July	30 August	331	224	67·6	132·1
	12 August	14 September	298	129	43·2	64·4
	24 August	25 September	304	39	12·8	34·8
Montgomery, 1939	15 July	14 September	1721	1256	73·0	106·5
	30 July	30 September	992	466	46·9	59·0
	15 August	15 October	1245	467	37·5	61·0
	30 August	30 October	1313	542	41·0	73·0
Beas, 1941	15 July	31 August	612	347	56·7	34·0
	31 July	16 September	583	200	34·7	27·4
	15 August	16 September	453	77	17·0	20·7
	1 September	16 October	565	52	9·2	8·1
Rawalpindi, 1942	22 July	15 September	571	97	17·0	80·4
	7 August	16 September	362	7	2·0	25·7
	22 August	31 October	651	45	7·5	35·5

It will be observed from the above table that the maize crop sown between 15 and 30 July suffered more from the pest than that sown between 7 August and 1 September.

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THE ONION THRIPS (*THRIPS TABACI* LIND.: THRIPIDAE: TEREBRANTIA: THYSANOPTERA)

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Thrips tabaci Lind. is nearly a cosmopolitan pest which has been recorded as occurring in such climatically different regions as South Europe, East and Central Europe, Asiatic Russia, India, Java, Japan, Australia, Hawaii, Chile, Mexico, U. S. A. and Canada. The presence of this pest in India was recorded for the first time by Karny in 1926 from the material collected by T. V. R. Ayyar as far back as March, 1920, from cotton flowers at Coimbatore while in the Punjab it was collected from Panipat, (District Karnal), in March, 1930, damaging onions and garlic. It was discovered at Lyallpur in 1935. Investigations on this pest were taken up seriously at Lyallpur in 1939 and this paper embodies the results of these investigations.

Life history

Thrips tabaci Lind. congregates in dense masses deep down in the narrow spaces between the axils of the inner leaves of the onion plant; it does not appear on the free portions of the leaves until present in very great numbers.

Oviposition. During March-June, two days after copulation, females started laying eggs. Eggs were laid singly in the leaf tissue on the underside of a leaf. A female laid 4-6 eggs per day and a total of 50-60 eggs in its life-time: according to MacGill [1927] a female laid 50-60 eggs in its life.

In leaves dehydrated in Alcohol and cleared in clove oil eggs could be seen as translucent spots under a binocular microscope.

The duration of the egg stage varied with the season: eggs laid in January-February hatched in 8-9 days while those laid in March, April, May and June took 7, 6, 5 and 4 days respectively to hatch. (For duration of egg stage in other countries see Table I below).

Nymphal instars. Eggs hatched out usually in the morning. The nymphal stage occupied 6 days during January-March and 4-5 days during April-June. (For duration of nymphal instars in other countries see Table I below.)

Pupation. Pupation took place in the soil throughout the year. The second instar nymph, when full fed, descended to the ground in which it burrowed to a depth of about an inch. Prepupal period occupied 1-2 days throughout the year while the pupal stage occupied 4 days during January-March, 3 days in April and 2 days in May-June.

Life-cycle. Table I gives the duration of the life-cycle of the pest in the Punjab and in other parts of the world.

Table I

Duration of life-cycle of T. tabaci *Lind. in the Punjab and other parts of the world*

Locality	Observer	Egg stage (days)	Larval stage (days)	Prepupal and pupal stages (days)	Total of immature stages (days)
Punjab	Rahman and Batra	4-9	4-6	3-5	12-19
Holland	Van Eecke	7	8	4	21-28
Massachusetts	Hind	4-7	7-8	7	21-28
Florida	Quaintance	3-4	7-9	4	16
Manchester	MacGill	8	10-14	5-9	23-31
Japan	Sakimura	3·6-6·3	6·4-14·0	4·5-10·3	14·5-30·3
U. S. A.	Eddy and Clarke	5	4-6	4·0-4·5	13·0-15·5

Seasonal history and food-plants

Seasonal history of *Thrips tabaci* Lind. was studied at Lyallpur during 1939-1941. The pest was found to appear on 'set onions', (grown from bulbs produced in the previous season) in the last week of November. It migrated from 'set onions' to 'seed onions' (grown from seed which are transplanted in January-February and harvested in June) in the beginning of spring when adults started laying eggs in large numbers. From about the beginning of March to the end of May all stages of the pest were found in the fields

and it was during this period that it did the greatest amount of damage to 'seed onions'. In June when the onions are harvested, the pest migrated to other food plants. Table II gives the food-plants of the pest monthwise.

TABLE II

Food-plants of T. tabaci *Lind. monthwise*

Month	Food-plant
October	1. Cabbage
	2. Cauliflower
November, December, January, February	3. 'Set onions' 4. Garlic
March, April, May, June	5. 'Seed onions'
July, August, September	6. *Karela* (*Momordica charantia*) 7. Chillies 8. Lady finger 9. *Petha* (*Benincasa cerifera*) 10. *Halwa Kadu* (*Cucurbita maxima*) 11. *Tori* (*Luffa aegyptica*) 12. *Til* (*Sesamum indicum*) 13. Cotton 14. Brinjal

In other parts of the world this insect has been recorded as feeding, in addition to 1-5 above, on the following food plants by various investigators: Parselay, cucumbers, melon, pumpkin, squash, turnip, tomato, lettuce, beets, beans, peas, celery, cotton, strawberry, blackberry, potato, sweet potato, lucerne, tobacco, spinach and wheat.

POPULATION SURVEY

A population survey of the pest on onions was carried out twice a week during November-May at Lyallpur and for this purpose 5 infested onion plants, which were selected at random from a field, were cut from the base with a sharp knife, enclosed in cellophane bags (measuring 12 in. × 6 in.) and brought to the laboratory, where they were carefully examined for nymphs and adults which were collected from them by unfolding the leaf blades one by one and afterwards counted under a binocular microscope. A record of rainfall as well as daily temperature was also kept. These data (lumped together from November to 3rd week of February and weekwise from 4th week of February to 2nd week of June) are presented in Table III.

TABTE III

Population of T. tabaci *Lind. on onions during November-June*

Month and week	Population per 5 plants	Temperature range in °F.	Rainfall in inches
November—3rd week of February	3-5	54·5-60·0	0·6
February IV	7	55·5-62·0	1·56
March I	12	57·0-64·5	0·66
II	26		
III	68		
IV	79		
April I	161	71·0-80·5	...
II	175		
III	220		
IV	180		
May I	126	86·0-92·0	...
II	82		
III	20		
IV	3		
June I	...	96·0	...
II	...		

PARTHENOGENESIS

Parthenogenesis is a common phenomenon among thrips and is also met with in *T. tabaci*, the parthenogenetically produced progeny consisting of females only. During March-June, 1940, 400 adults were examined and all of them were found to be females: Sakimura [1937] collected 5019 adults throughout the year in the vicinity of Tokyo from onions and found them all to be females.

DAMAGE

The injury which onion-thrips inflicts on the onion plant is called 'white blast', 'white blight' or 'silver tops', the whitened appearance of the onion leaves being due to the extraction from them of plant juices, first by rasping and then by sucking both by the adults and the nymphs. The attacked leaves become curled, crinkled and even twisted. The pest was found to do the greatest damage during dry seasons when it destroyed 75 per cent of the crop.

CONTROL

a. Sprays

Following insecticides, prepared according to the formula given against each, were tried against the pest during 1939 and 1940.

Tobacco decoction—

Tobacco refuse	1 lb.
Cheap soap	4 oz.
Water	1 gallon

Ak decoction

Ak (*Calotropis procera*)	2 lb.
Cheap soap	8 oz.
Water	3 gallons

Nim (*Melia azadirachta*) and *dhatura* (*Datura spc.*) decoctions prepared according to the above formula were also tested.

Rosin solignum soap

Rosin solignum soap	12 oz.
Water	4 gallons

Black leaf 40—

Black leaf 40	1 oz.
Fish oil soap	8 oz.
Water	6 gallons

Derrisol (Liquid katakilla)—

Derrisol	1 oz.
Fish oil soap	8 oz.
Water	5 gallons

Results are given in Table IV.

TABLE IV

Efficacy of different insecticides against T. tabaci

Serial number	Insecticide	Mortality percentage	Cost per acre
1	Tobacco decoction	75	Rs. 2
2	*Ak* decoction	61	Re. 1
3	*Nim* decoction	57	Re. 1
4	*Dhatura* decoction	52	Re. 1
5	Rosin solignum soap	66	Re. 1—12
6	Black leaf 40	82	Rs. 6
7	Derrisol	75	Rs. 8

It will be observed from the above table that Black leaf 40 gave a mortality of 82 per cent at a cost of Rs. 6 per acre.

It may be pointed out that a field of healthy onions is sold at Rs. 180-200 per acre but when infested with the onion thrips it may be sold for anything up to Rs. 25 per acre.

b. Dusts

Various dusts such as Nicotine dust (3 per cent), Tobacco dust (50 per cent), Naphthaline dust (33 per cent) and Crude Naphthaline were also tried against *T. tabaci*. These gave a mortality percentage of 26·8, 32·9, 14·1 and 18·5 at a cost of Rs. 8-8, Rs. 6-6, Rs. 2-8, and Rs. 16 per acre, respectively.

Maughan [1932] tried various dusts and sprays for the control of onion thrips (*Thrips tabaci* Lind.) on onions in Ithaca (New York) and found Nicotine preparations to be most effective in general, with kerosene, pyrethrum products, naphthaline and copper sprays or dusts giving high degrees of control.

SUMMARY

Thrips tabaci Lind. is a cosmopolitan insect which has been recorded as feeding on a large number of food plants. It is, however, a serious pest of onions.

In the Punjab, it completes its life-cycle in 12-19 days during the active period—January to June—as follows :

Egg stage, 4-9 ; larval stage, 4-6 ; prepupal and pupal stages, 3-5. Prepupal and pupal stages are passed in the soil at a depth of 1-2 in.

Onion thrips appears on ' set onions ' in November and remains on them till February : during this period it does little damage. It becomes active in March and its damage is at its highest during March-May. During June, it migrates to Malvaceous and Cucurbitaceous plants.

Parthenogenesis is a common phenomenon among these thrips, the parthenogenetically laid eggs producing females only.

The pest can be effectively controlled by spraying with Black leaf 40.

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VARIATION IN THE MEASUREABLE CHARACTERS OF COTTON FIBRES

VIII. VARIATION BETWEEN REGIONS OF THE SEED SURFACE

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Koshal and Ahmad [1932] have made detailed study on this point and find considerable differences among the different regions. Recently Moore [1941] also has observed similar variation. In Koshal and Ahmad's work the fibres on the whole seed were divided into four regions and examined. Besides, the seeds were obtained from the bulk sample. It was, therefore, thought interesting to study whether the variation observed would be reduced if a more uniform sample was chosen and if the fibres were taken from over a smaller region of the seed surface. This study is dealt with in the following pages.

Material and sampling

Four four-locked bolls picked from a single plant of Co. 2 on 14 March 1931 supplied the material for this enquiry. Out of this produce all the nine-seeded locks, with no undeveloped bolls, were separated. The seeds in the lock were numbered in the manner described elsewhere [Iyengar, 1941, 1] and sorted out according to the position of the seed in the lock. Thus nine main samples were obtained.

The surface of the seed was divided into six regions, namely (1) micropylar end (2) region close to the raphe (3) right side of the raphe (4) left side of the raphe (5) back of the raphe and (6) the chalazal end. The full halo was combed, care being taken to have as small a combed waste as possible. By means of dissecting needles fibres situated over the exact regions were separated and pulled out by hand. In order to avoid overlapping, bunches of fibres arising on the borderland between two contiguous regions were rejected. The fibres of the corresponding regions were put together and these formed the samples for examination. In some of the samples the raphe and back regions were not examined.

Experimental

The whole sample was made into a sliver and the mean fibre length was determined by means of the Balls sorter. As the sample was small only one Balls sorter test was made. The fibre weight per cm. was then determined by weighing 2,000 fibres cut to cm. lengths. (The cutting method was employed as the whole fibre-weighing-method was not in vogue when this work was done). The remaining portion of the sliver was used to determine the maturity percentage by using Clegg's [1932] method. Ten tufts of about 100 fibres each were examined for each sample.

Results

(a) *Variability of length*

Before taking up the mean length for each region the variability of the length in each region may be considered. In the present study this is assessed by the coefficient of variation of the length distribution as obtained by the Balls sorter.

The values of coefficient of variation given in Table I are the mean values. The individual values were found to vary from 14·0 to 25·1 per cent. The variation of the coefficient is considerable at the micropylar end. The mean value for this region is higher than for the others. The cause of the higher variability shown by the micropylar region may be the following. The mean fibre length for this region will be seen later to be considerably smaller than that for the other regions. If, therefore, instead of taking the fibres exactly over the micropylar end, it is taken over a slightly wider region a number of longer fibres enter into the sample and thus increase the variability. This effect is not prominent in the other regions as no such sharp variation in length occurs there.

The results considered above are for the different positions of the seed in the lock of one cotton, Co. 2. The coefficient of variation calculated from figures given by Koshal and Ahmad [1932] for four regions for eight cottons also indicate the same fact.

(b) *Fibre length*

The mean value for the micropylar region, 0·88 in., is significantly less than for the others. The right side and the left side having respectively mean lengths of 0·97 in. and 0·98 in. do not differ from each other. The mean length of

TABLE I

Fibre properties in the different regions of the seed

Property	No. of tests	Micropylar region	Chalazal region	Right side	Left side	Raphe* region	Back* region	Critical difference $P=0\cdot05$
1. Coefficient of variation of length per cent	9	20·5	18·6	16·3	15·7	19·0	15·1	...
2. Mean length (in.)	9	0·88	1·00	0·97	0·98	0·91	0·95	0·023**
3. Mean fibre wt. per cm. (10^{-6} gm.)	9	2·88	1·44	2·25	2·23	2·29	2·25	0·138**
4. ‡Mature fibres per cent.	9	88·0	30·1	76·6	72·1	86·0	69·0	... **
5. ‡Immature fibres per cent.	9	6·2	56·3	13·0	14·9	8·0	15·0	... **
6. Standard fibre wt. (10-6 gm.)	9	2·68	1·93	2·21	2·23	2·26	2·26	0·172**
7. Fibre cell diameter 2 days old (microns)	6	15·4±0·26	12·4±0·16	13·7±0·21	... ...	... ...	...	... ...
8. Fuzz diameter (microns)	4	30·3±0·81	25·3±0·68	25·6±0·72	... ...	... ...	...	... ...
9. Fibre diameter (microns)	3	25·6±0·43	21·2±0·34	22·1±0·27	... ...	... ...	...	... ...
10. Strength of attachment of the fibre to the seed (gm.) Co. 2	2	3·20±0·22	1·32±0·10	2·78±0·18	2·28±0·16	2·50±0·23	2·40±0·15	... ...
10. Strength of attachment of the fibre to the seed (gm.) K. C. 7	1	4·28±0·37	1·37±0·22	2·54±0·19	2·48±0·18	2·97±0·23	2·54±0·17	... ...

* The number of tests for properties 1 to 6 is only three for these two regions and the analysis of variance has not been applied to these regions

‡ For these properties the analysis of variance has been done using the inverse sine transformation

the chalazal region, 1·00 in., appears to be significantly higher than that for the right side and is nearly so than that for the left side. The mean for the raphe region, 0·91 in., indicates a smaller value but as it is based on three values only requires further confirmation. The mean length for the back region, 0·95 in., which is also derived from three values only, may not be significantly different from the right and left sides.

In another study [Iyengar, 1941, 2], only two regions, micropylar and chalazal, were studied for the same cotton, Co. 2. It is found that on the average the length is 0·88 in. for the micropylar and 1·00 in. for the chalazal end, coinciding with the mean values obtained above. The coincidence is, however, due to chance but it can be taken as an indication of good agreement.

The foregoing conclusions are in conformity with those drawn by Koshal and Ahmad [1932] and Moore [1941].

(c) *Fibre weight per cm.*

The mean value, 1·44, for the chalazal end is considerably less than that for the other regions. Similarly that for the micropylar end, 2·88, is significantly higher. The mean values for the other regions which vary from 2·23 to 2·29 do not indicate significant variation.

In the other study [Iyengar, 1941, 2], the mean fibre-weight for the micropylar end was got to be 2·86 and for the chalazal end 1·26, which are in substantial agreement with the present findings.

These results, like the length results, are in agreement with those of Koshal and Ahmad [1932] and of Moore [1941]. In some of the cottons studied by Koshal and Ahmad, however, the reduction in the fibre weight at the chalazal end is not as large as that found in the present work.

(d) *Fibre maturity*

Taking the mature fibres first it will be seen that the mean for the chalazal region, 30·1 per cent is very much smaller than that for the others. The mean for the micropylar end, 88·0, is higher than that for the right (76·6) or left (72·1) sides. No definite conclusion can be drawn regarding the raphe and back regions, as the mean values are based on three positions only. The immature fibres also confirm the previous statements. The chalazal region with 56·3 is considerably more immature and the micropylar region with 6·2 less immature than the other regions.

The results obtained for 14 strains in another study [Iyengar, 1941, 2] point to the same conclusion. In some of the strains, however, the variation in the different regions is not so considerable as observed in the present case. Moore [1941] and also Campbell, quoted by him, have also observed the same kind of variation.

Moore [1941], however, has attempted to correlate the variation in the fibre weight and

TABLE II

Number of fibres and nutrition supplied to the chalazal and micropylar regions

(Calculated from the figures given by Moore)

No.	Cotton	Number of fibres in 3·567 sq. mm.			Nutrition gm./cm²			B/A
		Ch.	Mic.	Ch./Mic. A	Ch.	Mic.	Ch./Mic. B	
1	Mexican 128	1,457	317	4.6	16.7	5.5	3·0	0·65
2	Coker Cleveland 884-4	2,044	241	8·5	19·1	4·1	4·7	0·55
3	Farm Relief No. 1	1,493	256	5·8	16·1	4·7	3·5	0·60
4	Acala 4067	1,646	406	4·1	15·7	6·2	2·7	0·66
5	Rowden 40	1,725	216	8·0	19·3	3·7	5·2	0·65
	Mean	1,673	287	5·8	17·4	4·9	3·5	0·60

maturity in the different regions with the number of fibres produced per unit area of the region. For the sake of simplicity two regions (1) and (6) of his study, which correspond to the chalazal end and the region close to the micropylar end may be considered here. The number of fibres produced in the punched area is given by him. The quantity of nutrition supplied can be got by multiplying the number of fibres by the corresponding mean length of the fibres and the fibre weight per unit length. The values thus obtained are given in Table II. They indicate that the number of fibres produced at the chalazal end is relatively very much greater—4·1 to 8·5 times the number near the micropylar region. The quantity of nutrition supplied is also considerably higher in the former region but not as great as observed in the number of fibres, being only 2·7 to 5·2 times. Thus each fibre at the chalazal end receives relatively less nutrition than at the micropylar end, being on the average about three-fifths. This accounts for the lower fibre weight per unit length and lesser degree of maturity shown by the fibres produced in the former region. The cause of the reduced nutrition appears to be, as shown by Moore, the relatively large amount of intermicellar space between the vein and the epidermal layer at the chalazal region.

e) *Standard fibre weight*

The mean value for the micropylar region, 3·68, is significantly more than that for the other regions. Similarly, the value for the chalazal end, 1·93, is significantly less. The variation among the other regions, 2·21 to 2·26, is not significant. The increase at the micropylar end over that at the chalazal end is 39 per cent of the latter value. The increase in the case of the actual fibre weight per cm. is found to be 100 per cent. In the other study [Iyengar, 1941, 2] the corresponding increases were found to be 44 and 127 per cent respectively. It means that the variation in the maturity or in the secondary thickening accounts for about two-thirds of the variation observed in the fibre weight, the remainder being due to the differences in the diameter of the fibre cell. From the above it follows that this diameter is largest at the micropylar end, smaller at the sides and still smaller at the chalazal end.

The results obtained for the actual uncollapsed diameter in another study [Iyengar, 1943] confirm the above findings. This feature appears to be true even in ovules which are very young. The diameter of the sprouting cell was determined for ovules of Co. 2, two days old, grown at Coimbatore. Three regions, micropylar end, right side and chalazal region have been studied in each case. At exactly the micropylar end no fibres are formed at the age of two days. Hence the diameter of the fibres nearest that region was determined. Six ovules have been studied and the results show that in each case the diameter for the micropylar region (15·4) is greater than that for the right side (13·7) which in turn is greater than that for the chalazal end (12·4), just as is found in mature seeds.

The diameter of the fuzz fibres also signifies a similar tendency. The fuzz was swollen in 18 per cent caustic soda and about 100 to 170 fibres were measured for each region of a seed. Four seeds of Co. 2 (fifth seed of nine-seeded locks) were examined. The results denote that in all cases the micropylar region (30·3) indicates a significantly higher value as was found in the

case of lint fibres. But the difference between the chalazal region (25·3) and the right side (25·6) is not significant.

The diameter of fuzz is greater than that of lint in all cases agreeing with the finding of Balls [1928] and of Brown [1938]. But the other statement of Brown that as the cell wall is thin, they resemble immature or undeveloped fibres is not borne out. The results obtained for Co. 2 cotton show that the maturity of the fuzz from all over the seed is 95—3. Even at the chalazal end, where the lint fibres were found to be very immature, the maturity of the fuzz is 84—12, while for lint from the same seeds it is 42—46. In another sample the maturity of fuzz at the chalazal end was found to be 94—4 as against the maturity of lint of 57—29. It is thus seen that the fuzz fibres are considerably mature agreeing with the statement of Balls [1928] that these fibres show restricted length-growth with rank thickness-growth.

(f) *Strength of attachment of the fibre to the seed*

This is a new property studied in this connection. It was measured by finding the force required to pull out the fibre by loading it by means of the O'Neill float. The seed was held in a padded clamp and the other end of the fibre to which an eyelet was fixed supported the float. Thirty fibres were pulled out for each region of the seed. The six regions described above have been studied in this case also. For this work a small tuft sprouting exactly on the required region was separated by means of a needle. Fibres from this region were mounted one after another and pulled out and the weight required for the pull was determined.

Two seeds of Co. 2 and one of Karunganni C. 7 have been examined. Unfortunately the work could not be extended to more samples on account of various causes. The results obtained indicate that the strength of attachment of the fibre to the seed is greatest at the micropylar end, least at the chalazal end and intermediate in the other regions, the differences among which are not conspicuous. The low strength at the chalazal end may be due to the fact revealed in Plate 2 of Moore's [1941] work. The structure of the cells at the chalazal end is not quite uniform and there is considerable intermicellar space, while in the other region the structure is more uniform with a relatively small amount of intermicellar space.

Discussion

Summing up the foregoing it may be stated that (1) at the micropylar region the fibres are thinly populated, short, very mature, have the highest fibre weight, standard fibre weight, fibre diameter, fuzz diameter and fibre strength and are most firmly attached to the seed; (2) at the chalazal end the fibres are very densely populated, are longer and more immature—considerably more immature in some cottons—have smaller fibre weight per cm., standard fibre weight, fibre diameter and fibre strength and are less firmly attached to the seed; and (3) at the other regions the variations among themselves do not appear to be conspicuous; generally speaking the values lie intermediate between those for the two regions considered above though they approach the values for either of the two regions in some cases.

The probable cause for the variation observed in the different regions may now be considered. On this point Balls [1928] says 'No doubt it is largely a question of nutrition, dependent upon the anatomy of the vascular bundles on the seed coat.' Talking about the cross-sectional area of the fibre he states that 'The proximate causes for this variation can be traced to the life history of the hair. It depends only slightly upon the variations in the original diameter of the cell. Principally it is brought about by the difference in the thickening of the secondary wall.' The present results in the case of the micropylar and chalazal ends of the seed indicate that about two-thirds of the weight is accounted for by secondary thickening, the rest one-third being due to the original cell diameter. Moore's [1941] findings of the venations on the seed-coat and of the differences in the structure of the cells below the epidermis in the different regions offer a partial explanation. Further critical enquiry is indicated.

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STATISTICAL NOTES FOR AGRICULTURAL WORKERS

No. 27. CALCULATION OF STANDARD ERRORS AND TESTS OF SIGNIFICANCE OF DIFFERENT TYPES OF TREATMENT COMPARISONS IN SPLIT-PLOT AND STRIP ARRANGEMENTS OF FIELD EXPERIMENTS

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(With two text-figures)

In agricultural field experiments conducted to study simultaneously the effects of variation of more than one factor, research workers find practical difficulties in completely randomizing all the treatments within a single block. To remedy this operational difficulty two designs called Split-Plot and Strip Arrangements have been devised which have become quite popular among agricultural workers. But from the statistical point of view of precision of treatment comparisons these designs suffer from certain drawbacks. Thus, the more important comparisons called main effects are less precisely estimated than interactions.

Tests of significance of comparisons which involve only one of the main effects or of the interactions are quite straightforward and reduce to t-test. But sometimes even in factorial experiments one may like to compare any two treatment combinations and the calculation of their standard errors is a little complicated and the method is not widely known. As a result many are not aware of the fact that the test of significance of these comparisons cannot be made by Student's t-test. It will be shown in the note that the Behrens and Fisher's test for which Sukhatme [1938] has published convenient tables can be applied for certain comparisons and that there are also comparisons for which no exact test of significance is known.

Strip arrangement

Let us consider a two factor experiment with p variants, $a_1, a_2, \cdots a_p$ of a factor A and q variants $b_1, b_2, \ldots b_q$ of a factor B in all combinations each replicated in r blocks. Each block is divided into p column strips to which $a_1 \cdots a_p$ are assigned at random; and divided into q row strips to which $b_1 \ldots b_q$ are assigned at random. Such a lay-out is called a Strip Arrangement. Fig 1 represents a typical block. It will be noted that there is no free randomization for each treatment combination $a_i\, b_j$ within the block.

The structure of the analysis of variance of plot yields is shown in Table I.

The standard errors σ_0, σ_1, σ_2, σ_3 are those associated with plots of the size and shape represented in Fig. 1 by AEKD, ABCD, AEFG and ABHG respectively. If the yields of all the pq plots of size A B H G were all uncorrelated, it is easy to show that $\sigma_0 = \sigma_1 = \sigma_2 = \sigma_3$. In other words the standard errors associated with different plot sizes when they are all reduced to the same scale can be the same in only 'random' fields. It is a very widely verified fact that adjacent plots of a field are highly and positively correlated owing to certain regularity in fertility variation.

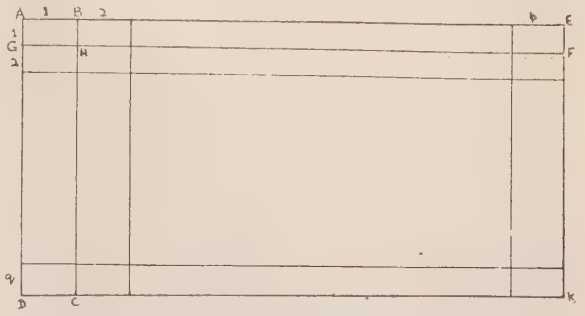

Fig. 1. One block of a strip arrangement

Table I

Analysis of Strip Arrangements

	D. F.	Variance	
		Observed	Expressed under nullhypothesis
Blocks	$r-1$	V_0	σ_0^2
Main effect : A	$p-1$	Va	σ_1^2
Error (1)	$(p-1)(r-1)$	V_1	σ_1^2
Main effect : B	$q-1$	Vb	σ_2^2
Error (2)	$(q-1)(r-1)$	V_2	σ_2^2
Interaction : A × B	$(p-1)(q-1)$	Vab	σ_3^2
Error (3)	$(p-1)(q-1)(r-1)$	V_3	σ_3^2
Total	$pqr-1$		

Based on this phenomenon it is possible also to build up an abstract theory about the quantitative behaviour of these correlations as has been put forward by Mahalanobis [1942], who uses the term 'correlation function' to describe this relationship. In his theory, all pairs of plots at an equal distance may be assumed to give one value for the correlation, whatever be the direction of the line joining the centres of the two plots. By exhausting all pairs of plots situated at distance d_1, d_2, ... d_n we can get n values of correlation, r_1, r_2, ... r_n. Plotting d and r along x and y axes we get a graph of the correlation function which may then be suitably graduated. The general shape of the correlation function with which we come across in agricultural field experiments is shown in Fig. 2.

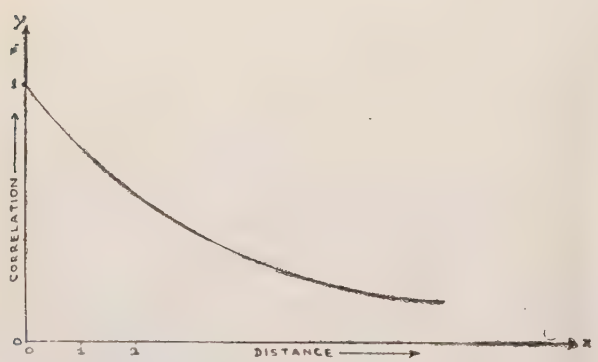

FIG. 2. Correlation function for agricultural fields

The correlation is obviously + 1 at distance 0 but decreases to 0 as distance between pairs of plots increases indefinitely. The correlation never becomes negative if sufficient pairs of plots are included.

Having accepted the above form of the correlation function, it simply follows that σ_1 and σ_2 must be less than σ_0 and greater than σ_3. The difference between σ_1 and σ_2 will mostly depend on difference in area between plots ABCD and AEFG, that is, on the difference between p and q, but will also be controlled by the 'differential' shape of these plots which also depends on p and q. They will be very dissimilar in shape if the plot ABHG gets narrower in shape. If $p = q$, the shapes will be the most important factor influencing the relative magnitude of σ_1 and σ_2. It follows from the form of the correlation function shown in Fig. 2 that narrow plots are less variable among themselves than square ones of the same area. This is for the obvious reason that in the former plots there are proportionately more pairs of distant components [or *quads* as Mahalanobis (1942) calls them] with small correlations than in the latter plots. Thus, in our case, σ_1 will be greater than σ_2 when $p=q$, if AB is greater than AG and *vice versa*.

Whatever be the relative magnitudes of σ_1, σ_2 and σ_3, it is enough to note that they are not likely to be equal. We have to make separate estimates of them, namely, the square roots of V_1, V_2, V_3 of Table I. The observed variances Va, Vb and Vab for main effects of A of B and of interaction A $\times$ B respectively should be tested against V_1, V_2, V_3 by the 'Ratio of Variance' test.

Besides the above analysis of variance it is necessary to make detailed comparisons between pairs of treatment combinations of which there are three main types namely :

(*i*) Comparison between variants of B for a given variant of A, Ex. $a_1\,b_1$—$a_1\,b_2$.

(*ii*) Comparison between variants of A for a given variant of B, Ex. $a_1\,b_1$—$a_2\,b_1$.

(*iii*) Comparison not included in (*i*) and (*ii*), Ex. $a_1\,b_1$—$a_2\,b_2$.

These comparisons can be expressed in terms of the two main effect comparisons of the type $\left(\sum_1^q b_i\right)(a_1-a_2)$ and $\left(\sum_1^p b_i\right)(b_1-b_2)$ and interaction comparisons of the type (a_1-a_2) (b_1-b_2) which are mutually orthogonal and whose standard errors depend on σ_1, σ_2 and σ_3 respectively. To calculate the variance of a_1b_1-a_1b_2 which may be denoted by $V(a_1b_1-a_1b_2)$, we must proceed as follows :

$$p\,(a_1\,b_1-a_1b_2) = \left(\sum_1^p a_i\right)(b_1-b_2) + [(a_1-a_2) + \ldots + (a_1-a_p)]\,(b_1-b_2) \quad \ldots\ldots\ (1)$$

It may thus be noted that a_1b_1—a_1b_2 splits into two component parts, one involving main effect of B and the other the interaction A $\times$ B. The two parts are obviously orthogonal and therefore

$$p^2V\,(a_1\,b_1-a_1\,b_2) = V[\,(\Sigma a_i)\,(b_1-b_2)] + V\left[\left\{(p-1)\,a_1-a_2-\ldots-a_p\right\}(b_1-b_2)\right] \quad \ldots\ (2)$$

If the comparisons are estimated on a per plot basis from r replications

$$p^2\,V\,(a_1\,b_2-a_1\,b_2) = \frac{2\,p}{r}\,\sigma_2^2 + \frac{2p(p-1)}{r}\,\sigma_3^2$$

$$or\ V\,(a_1\,b_1-a_1\,b_2) = \frac{2}{r}\left(\frac{\sigma_2^2+(p-1)\sigma_3^2}{p}\right) \quad \ldots\ (3)$$

Similarly, to calculate the variance of $a_1\,b_1$—a_2b_1 we have

$$q\,(a_1\,b_1-a_2\,b_1) = \left(\sum_1^q b_i\right)(a_1-a_2) + [(b_1-b_2)+\ldots+(b_1-b_q)\,]\,(a_1-a_2) \quad \ldots\ (4)$$

$$and\ q^2\,V\,(a_1\,b_1-a_2\,b_1) = \frac{2q}{r}\,\sigma_1^2 + \frac{2q\,(q-1)}{r}\,\sigma_3^2$$

$$or\ V(a_1 b_1 - a_2 b_1) = \frac{2}{r}\left(\frac{\sigma_1^2 + (q-1)\sigma_3^2}{q}\right) \quad .. \quad (5)$$

Coming now to the third type of comparison, namely, $a_1 b_1 - a_2 b_2$, we have

$$2(a_1 b_1 - a_2 b_2) = (a_1 + a_2)(b_1 - b_2) + (a_1 - a_2)(b_1 + b_2) \quad .. \quad .. \quad .. \quad .. \quad .. \quad (6)$$

$$and\ 4\ V(a_1 b_1 - a_2 b_2) = V[(a_1+a_2)(b_1-b_2)] + V[(a_1-a_2)(b_1+b_2)] \quad .. \quad .. \quad .. \quad .. \quad (7)$$

To calculate each of the variances on the r. h. s. of (7) :—

$$p(a_1+a_2)(b_1-b_2) = 2\left(\sum_1^p a_i\right)(b_1-b_2) + [(p-2)(a_1+a_2) - 2\sum_3^p a_i](b_1-b_2) \quad .. \quad .. \quad .. \quad (8)$$

$$p^2 V[(a_1+a_2)(b_1-b_2)] = \frac{8p}{r}\sigma_2^2 + \frac{4p(p-2)}{r}\sigma_3^2$$

$$or\ V[(a_1+a_2)(b_1-b_2)] = \frac{4}{r}\left(\frac{2\sigma_2^2 + (p-2)\sigma_3^2}{p}\right) \quad .. \quad .. \quad .. \quad (9)$$

Similarly

$$V[(a_1-a_2)(b_1+b_2)] \frac{4}{r}\left(\frac{2\sigma_1^2 + (q-2)\sigma_3^2}{q}\right) \quad .. \quad (10)$$

In general

$$V[(a+a_2+\ ..\ +a_k)(b_1-b_2)] = \frac{2k}{r}\left(\frac{k\sigma_2^2 + (p-k)\sigma_3^2}{p}\right) \quad .. \quad .. \quad .. \quad (11)$$

$$V[(b_1+b_2+\ ...\ +b_k)(a_1-a_2)] = \frac{2k}{r}\left(\frac{k\sigma_1^2 + (q-k)\sigma_3^2}{q}\right) \quad .. \quad .. \quad (12)$$

When k equals p and q respectively, σ_3 drops out from (11) and (12) as it should be.

Adding (9) and (10) we have

$$V(a_1 b_1 - a_2 b_2) = \frac{2}{pqr}\left(p\sigma_1^2 + q\sigma_2^2 + (pq-p-q)\sigma_3^2\right) \quad .. \quad (13)$$

Tests of Significance. Both $a_1 b_1 - a_1 b_2$ and $a_1 b_1 - a_2 b_1$ are quantities whose standard errors are measured by estimates of two unequal variances and hence not capable of being tested by Student's t.

Similarly comparisons whose variances are given by (11) and (12) cannot also be tested by Student's t unless $k=p$ in (11) and $k=q$ in (12) in which case they reduce to main effect comparisons. For other values of k we can apply the Behrens and Fisher test which Sukhatme [1938] calls d-test. Thus taking (11), we have

$$d = \frac{(a_1+a_2+\ ...\ +a\quad (b_1-b_2)}{\sqrt{\frac{2k}{rp}\left\{kV_2 + (p-k)V_3\right\}}} \quad (1 \leqslant k < p) \quad (14)$$

and we have to enter Sukhatme's tables with $n_1 = (q-1)(r-1)$, $n_2 = (p-1)(q-1)(r-1)$ and θ given by $\tan\theta = \sqrt{kV_2/(p-k)V_3}$.

Similarly for (12)

$$d = \frac{(b_1+b_2+\ ..+b_k)(a_1-a_2)}{\sqrt{\frac{2k}{rq}\left\{kV_1 + (q-k)V_3\right\}}} \quad (1 < k < q) \quad (15)$$

with $n_1 = (p-1)(r-1)$, $n_2 = (p-1)(q-1)(r-1)$ and $\tan\theta = \sqrt{kV_1/(q-k)V_3}$.

The assumption in the d-test is that neither the two population variances nor their ratio are known, but in our experiment we have an almost certain knowledge which of the two variances is greater so that we can set the boundary for the ratio of variance as always lying above 1. But this information is not being utilized by the d-test. Yates [1939] has made a careful study of the d-test from which it appears that by not taking into account the *a priori* knowledge of the range of values of the unknown ratio of variances, the proportion of samples judged significant will be less than the actual level of significance adopted would indicate.

Coming now to the last type of comparison namely, $a_1 b_1 - a_2 b_2$, its standard error involves three unequal standard deviations σ_1, σ_2 and σ_3. Neither the t nor the d test is applicable to it. Since $(a_1 + a_2)(b_1 - b_2)$ and $(a_1 - a_2)(b_1 + b_2)$ have to be tested by d, we can look at $a_1 b_1 - a_2 b_2$ as a compound of two d values, just in the same way as d is a compound of two t values, but there is no known method of testing its significance.

Split-plot arrangement

If we had split-plot design instead of the above strip arrangement, σ_2 and σ_3 will stand for the same standard deviation if the variants of factor A occupy the main plots ABCD, etc., and the q variants of B are perfectly randomized among q sub-plots, ABHG, etc. of each main plot. The effect of any particular variant of B will no longer be estimated from compact strips of the type AEFG. The new σ_2 which measures the uncontrolled fertility variation between plots of the type ABHG within main plots of the type ABCD, can be estimated with $p(q-1)(r-1)$ degrees of freedom by pooling Errors (2) and (3) of Table I. The new V_2 can be used to test the significance of both Vb and Va. The variances of the 3 types of elementary comparisons considered before can be obtained by putting $\sigma_2 = \sigma_3$ in (3), (5) and (13). The altered results are

$$V(a_1 b_1 - a_1 b_2) = 2\sigma_2^2/r \quad .. \quad (16)$$

$$V(a_1 b_1 - a_2 b_1) = V(a_1 b_1 - a_2 b_2) = \frac{2}{r}\left(\frac{\sigma_1^2 + (q-1)\sigma_2^2}{q}\right) \quad (17)$$

It is easy to see that $a_1 b_1 - a_1 b_2$ can be tested by t with d. f. $= p(q-1)(r-1)$. But $a_1 b_1 - a_2 b_1$ and $a_1 b_1 - a_2 b_2$ can be tested only by d test for which Sukhatme's table should be entered with $n_1 = (p-1)(r-1)$, $n_2 = p(q-1)(r-1)$; and θ given by $\tan\theta = \sqrt{V_1/(q-1)V_2}$, V_2 being estimated by pooling the Errors (2) and (3) of Table I.

NUMERICAL EXAMPLE

For sake of illustration, I am giving below certain results taken from the previous Note (No. 26, of this series by S. Hedayetullah, S. Sen and K. R. Nair. We shall discuss the portion relating to varieties and dates of planting only of the year 1937-38, when we got significant values for the interaction between varieties and dates of planting. The mean yields for each variety and date of planting are given in Table II, figures excepting those in the margins being each based on average of 9 plots.

TABLE II

Mean yield of paddy per plot (9 ft. × 20 ft.) in tolas (Dacca Experiment, 1937-38)

Variety	Date of planting						Mean
	1 July	16 July	1 August	16 August	1 September	16 September	
1	728·44	780·00	698·44	540·00	406·89	354·44	584·70
2	618·22	592·22	618·44	473·33	460·67	256·00	503·70
3	584·44	576·44	549·33	451·56	406·22	203·55	461·93
Mean . .	643·70	649·56	622·07	489·41	424·59	271·33	516·78

The values of observed variances for each main effect and the interaction are given in Table III.

TABLE III

Results of analysis of variance

	D. F.	Variance	Ratio of variance
Blocks . . .	2	72258·67	×
Varieties . .	2	210426·89	5·47
Error (I) . .	4	38492·11	×
Dates of planting .	5	617315·36	25·27†
Error (II) . .	10	24433·10	×
Varieties × Dates .	10	17287·81	2·55*
Error (III) . .	20	6778·20	×

* Indicates significance at 5 per cent point
† Indicates significance at 1 per cent point

It will be noted that Error (I) is greater than Error (II) and both much greater than Error (III). The first error is associated with the varietal strips and the second error associated with the strips for dates. The former strips are double the latter ones in size and hence Error (I) has become greater than Error (II).

Standard error for comparing yields of any two dates for a given variety is

$$\sqrt{\left[\frac{2}{9}\left(\frac{24433\cdot10+2\times6778\cdot20}{3}\right)\right]} = 53\cdot05$$

For $n_1 = 10$, $n_2 = 20$, $\tan\theta = \sqrt{\frac{24433\cdot10}{13556\ 40}}$

$= 1\cdot80$ or $\theta = 60°\ 59'$

the value of $d_{5\%}$ from Sukhatme's Tables is 2·188.

A difference of $53\cdot05 \times 2\cdot188 = 116\cdot07$ in the yield between two dates for a given variety should be judged as significant on the 5 per cent level.

Standard error for comparing yields of any two varieties for a given date is

$$\sqrt{\left[\frac{2}{9}\left(\frac{38492\cdot11+5\times6778\cdot2}{6}\right)\right]} = 51\cdot78$$

The smallest value of n_1 tabulated in Sukhatme's Tables is 6. But in our case $n_1 = 4$, $n_2 = 20$, $\tan\theta = \sqrt{\frac{38492\cdot11}{33891\cdot00}} = 1\cdot14$ or $\theta = 48°\ 38'$. The value of $d_{5\%}$ will be greater than 2·280 which is the value for $n_1 = 6$, $n_2 = 20$ and $\theta = 48°38'$.

In the present experiment the comparisons in which we are more interested are those which describe the properties of the yield-date curve for each variety, which may be graduated by a second degree parabola

$$y = A + B\xi_1 + C\xi_2 = a + bx + cx^2 \quad \ldots \quad (18)$$

where x = date of planting and y = mean yield per plot.

The values of the parameters involved in (18) are given in Table IV separately for each variety.

TABLE IV

Values of coefficients of the yield-date curve

Variety	A	B	C	*a*	*b*	*c*
1	584·70	—89·94	—12·97	778·88	0·84	—12·97
2	503·70	—67·07	—18·97	562·06	65·70	—18·97
3	461·93	—71·80	—18·68	539·47	58·98	—18·68

Standard errors of B and C are respectively given by

$$\sqrt{\left[\frac{2}{9\times35}\left(\frac{24433\cdot10+2\times6778\cdot20}{3}\right)\right]}=8\cdot96\ ;$$

and

$$\sqrt{\left[\frac{1}{336}\left(\frac{24433\cdot10+2\times6778\cdot20}{3}\right)\right]}=6\cdot14.$$

The value of $d_{5\%}$ for testing the significance of B and C is 2·188. The significant values of B and C must be greater than 19·60 and 13·43 respectively. We find that all the 3 values of B are highly significant; and that only the value of C got for variety 1, is just insignificant.

The standard error of the difference between any two values of B and any two values of C are given respectively by

$$\sqrt{\frac{4}{9\times35}\times6778\cdot20}=9\cdot28 \text{ and } \sqrt{\frac{1}{168}\times6778\cdot20}=6\cdot35$$

These differences can be tested by t. Values of $t_{5\%}$ for 20 degrees of freedom is 2·086. The critical differences for the B's and for the C's are 19·36 and 13·25 respectively. We find that the quadratic coefficients do not differ significantly but linear coefficients for varieties 1 and 2 are significantly different.

In conclusion, my thanks are due to Mr S. Raja Rao for help in the numerical work.

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DETERMINATION OF SODIUM BENZOATE IN PRESERVED CITRUS JUICES AND SQUASHES

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(With one text-figure)

SODIUM Benzoate is one of the preservatives commonly used in the preservation of juices and squashes. Its determination is consequently of great importance in the analysis of these products. A concentration of a thousand parts per million is permitted: but in practice, specially if used in combination with sulphur dioxide, it is seldom in excess of 500 p.p.m. In the course of determination of this preservative in some samples of citrus juices and squashes, sent to this laboratory by a leading firm of manufacturers, it was found that determination in concentrations of the order mentioned is not as accurate as would be desired for the purpose in view.

In the official A.O.A.C. method [1940] the sample is made alkaline to litmus and extracted with saturated salt solution. The residue is allowed to settle and the clear extract filtered. The filtrate is acidified and extracted with chloroform in a separating funnel to get the benzoic acid which is determined by titration against standard alkali, after evaporating the solvent. In practice this method was found to give too low results, which did not agree between themselves. For example, the samples mentioned above were supposed to contain 500 to 600 p.p.m. But the results in several replicates varied from 40 to 400 p.p.m. To ascertain whether any appreciable amount of the sodium salt is absorbed on the fruit cells, even in alkaline solution, a 100 gm. sample of orange juice containing 890 p.p.m. of benzoic acid was thoroughly ground with sand, salt being added at

the same time to saturate it, as directed in the A.O.A.C. method. This sample gave only 396 p.p.m. In case of unground sample the recovery was 320 p.p.m. The residue left on the filter paper in the latter case was ground with sand and washed with 150 c.c. water; but only an equivalent of 37 p.p.m. of benzoic acid was recovered from the filtrate. It is, therefore clear that the A.O.A.C. method is not suitable for the determination of benzoic acid used as a preservative in citrus juices and squashes. Other methods to which reference may be made are those of Illing [1932] and Mix [1939]. These depend on the volatility of benzoic acid in boiling acid solutions. But, on his own showing, Mix found a high variation (+16 per cent to —4 per cent) even in water solutions of sodium benzoate. The extraction is far more complicated in case of fruit juices and squashes when sugar and fruit cells are present in the mixture.

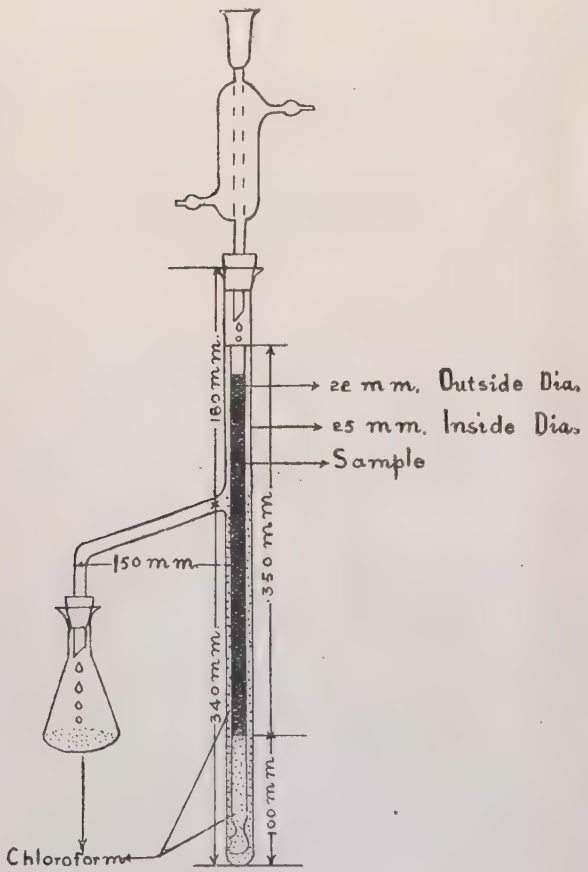

FIG. 1

A modified extraction method was, therefore, tried in which the apparatus shown in Fig. 1 was used. This apparatus is suitable for extracting 100 c.c. of the sample. It can be held in two clamps mounted on the same stand, thus facilitating removal from and to the water bath. The apparatus is charged with chloroform and 100 c.c. of sample acidified with 3 c.c. of strong hydrochloric acid are introduced into the inner tube through the long stemmed funnel. The chloroform rises into the outer jacket and some falls into the boiling flask. If necessary, more chloroform may be added through the same funnel. The reflux condenser is now fitted and the flask kept for boiling on a water bath. The boiling should be brisk so that the falling drops serve to shake the sample. If, as often happens, the fruit cells settle into a solid mass, this should be broken up once or twice with a long thin glass rod with a flattened end. As regards time of extraction it was found that two hours are not sufficient. Four and eight hours gave almost similar results and four hours are therefore recommended. The chloroform column below the sample should be at least 8 cm. long, otherwise there is risk of some droplets of the emulsion being carried to the boiling flask. At the end of the extraction the apparatus is taken off the water bath, cooled and the boiling flask removed. The sample is removed by suction through a long thin glass tube and the apparatus is ready for another extraction. The extract in the boiling flask is transferred to a separating funnel and washed with a little water, the flask and washing water being again washed with two portions of 5-10 c.c. chloroform. The chloroform solution and washings are collected in a flask and the solvent recovered by distillation till about 15 c.c. of the solution is left in the flask. This is transferred to a glass dish and the flask washed with 10 c.c. chloroform in two portions and the dish allowed to lie under a fan to evaporate the solvent. The residual benzoic acid is dissolved in neutral alcohol (85-90 per cent) and titrated with standard alkali, using phenelphthalein as indicator.

To test the accuracy of the method a sample of orange juice was separated into lots in which different amounts of sodium benzoate were added. Results of the replicate determinations of these samples are given in Table I.

Some of these juice samples and two samples of orange squash were analysed by both methods. The results in Table I show that whereas the A.O.A.C. method gives very low results, the method recommended gives only a slight variation. Statistical analysis of the results showed that the variation was not significant, and that the accuracy is unaffected by the change of concentration from 45 to 1800 p.p.m., the reaction being non-significant.

TABLE I

S.No.	Description of sample	No. of replications	Sodium benzoate mg. per litre (p.p.m.)		Method used
			Taken	Found (average)	
1	Prepared in the laboratory by adding known amounts of sodium benzoate to fresh orange juice	2	44	39	New method
2	Ditto.	3	89	79	Do.
3	Ditto.	4	178	177	Do.
4	Ditto.	2	267	300	Do.
5	Ditto.	3	356	380	Do.
6	Ditto.	6	445	462	Do.
7	Ditto.	2	535	523	Do.
8	Ditto	3	668	627	Do.
9	Ditto.	4	713	712	Do.
10	Ditto	3	890	914	Do.
11	Ditto.	2	1340	1335	Do.
12	Ditto.	2	1780	1687	Do.
13	Ditto.	2	1780	1082	A.O.A.C.
14	Ditto.	2	890	320	Do.
15	'A' orange squash received from firm of manufacturers	2	500 to 600	352	Do.
16	'B' " " " "	2		324	Do.
17	'A' " " " "	2		540	New method
18	'B' " " " "	2		604	Do.

SUMMARY

The official A.O.A.C. method for the determination of benzoic acid is shown to be inaccurate for preserved citrus juices and squashes. A modified method is described in which the samples are placed in a long column and extracted for four hours. The formation of emulsion is avoided. The results obtained by this method are far more accurate and are not subject to variation due to change of concentration.

ACKNOWLEDGEMENT

The author is deeply indebted to Sardar Bahadur Sardar Lal Singh, Fruit Specialist, Punjab, under whose guidance the work was carried out.

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STANDARDIZATION OF CURRY POWDERS

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(Received for publication on 13 May 1944)

Analysis of several samples of curry powders, received in these laboratories from the Supply Department, showed that good deal of precise information on granularity, chemical standards and bacterial standards was necessary since general methods used in their preparation are very empirical. It was, however, recognized that rigid standards cannot be fixed unless extensive investigations were undertaken on condiments grown in different localities. Nevertheless, a few samples were analysed and have yielded interesting preliminary results. These results are presented under three heads, viz. (*a*) Physical test of granularity, (*b*) Chemical analysis, and (*c*) Micro-biological analysis.

Material and methods

Six different samples of curry powders were obtained from different firms and were claimed by them to conform to the following recipe :

	Parts
(1) Turmeric	40
(2) Mustard	5
(3) Coriander	20
(4) Pepper (black)	5
(5) Chillies	10
(6) Common salt	3
(7) Cumin seeds	5
(8) Cloves	2
(9) Cinnamon	5
(10) Ginger	5

In addition to these six samples, a sample was prepared in the Laboratory and will be referred to in the text as 'Ls.'

Methods used in the analyses except in the case of physical test of granularity were standard methods [A. O. A. C., 1940 ; Tanner, 1932]. In the case of the granularity test, a modification of the technique used for cereal flours [Sterckx, 1939] was employed with success and will be described in detail under that head.

Experiments and results

A. *Physical test of granularity*

Two methods are usually employed for determination of the degree of granularity in cereal flours, (*a*) the sieving test, and (*b*) the sedimentation test. The former is essentially a comparative test and especially in the case of curry powders presents a serious difficulty in that the substance chokes the bolting silks due to a high oil content. The latter method has been suggested by Sterckx [1939] wherein flour is suspended in a mixture of petroleum ether (40°—60°C. boiling point) 90 per cent and acetone 10 per cent and the mixture poured into a burette of stated dimensions containing the same liquid. The particles sink and stratify in different layers. Sterckx sums up by saying 'the method is open to considerable amount of experimental error when used by different operators'. This sedimentation method has been tried here in the case of curry powders with a few modifications. A 5 gm. sample of the curry powder contained in a shallow porcelain dish was suspended in about 10 c.c. of petroleum ether and acetone mixture (as given above) and quickly poured into an ordinary 50 c.c. (1/10 c.c.) burette containing a column of mercury in the bottom up to the 40 c.c. mark. The rest of the powder adhering to the dish was also washed down the burette with a fine jet of the mixture from a wash bottle. More of the mixture was poured into the burette till the level rose up to 0·0 c.c. The burette was left undisturbed for about an hour after which the reading in c.c. was taken of the different layers occupied by the different particles of powder. The line of demarcation between particles of different sizes was particularly clear between the 'coarse' and the 'fine particles', although with a little experience it was not difficult to distinguish between very coarse, coarse, fine and very fine particles. However, the result obtained in these experiments seem to be very consistent if viewed as to categories, viz. 'coarse' and 'fine'.

The use of a column of mercury as done here seems to be beneficial, firstly in that the particles are kept in a form of agitation as soon as they are poured in and not allowed to quickly settle down as happens in the case where no mercury is used and it is obvious that a speedy sedimentation of the coarse particles would influence that of the fine ones ; secondly the miniscus of the mercury permits of accurate reading of the burette. It may be emphasized that three precautions are necessary for consistent results ; firstly, the quick transference of the mixture and powder into the burette secondly the need for a burette with very clean walls and lastly, the temperature of the room should be far below that of the boiling point of the

petroleum-ether, otherwise large bubbles of air appear in the settling layers and adversely affect the result.

The results obtained with seven samples of curry powders are presented in Table I.

The results seem to permit of the following conclusions when 5 gm. samples were used for the test.

(*a*) Coarse and very coarse particles should have sedimentations volumes not less than 5·00 c.c. and not more than 7·00 c.c.

(*b*) Fine and very fine particles should have sedimentation volumes not less than 3·00 c.c. and not more than 4·00 c.c.

(*c*) The total volume occupied by any one sample should not exceed 10 c.c.

Sample No. 6 is definitely very coarse and does not conform to these standards. Similarly sample No. 3 has more of coarse and less of fine particles.

TABLE I

Granularity test of curry powders

Sample No.	No. of gm. of sample used for the test	Total No. of c.c. occupied by sample in burette	Sedimentation volumes in c.c. occupied by the different strata of sample	
			'Coarse particles'	'Fine particles'
1	5·0	9·85	6·25	3·60
2	5·0	9·75	6·00	3·75
3	5·0	10·15	7·55	2·60
4	5·0	9·20	5·55	3·65
5	5·0	9·85	6·75	3·10
6	5·0	15·10	10·55	4·55
Ls*	5·0	8·80	5·00	3·80

*Ls stands for Laboratory prepared sample

TABLE II

Chemical analyses of curry powder

Sample No.	Moisture per cent	Total ash per cent	Ash soluble in HCl per cent	Ash insoluble in HCl per cent	Alkalinity of ash as c. c. of N/10 HCl reqd. for ash of 100 gm.	Cold water extract per cent	Alcoholic extract per cent	Volatile ether extract per cent	Non-volatile ether extract per cent	Crude fibre per cent	Nitrogen per cent	Nx 6·25 per cent protein	NaCl per cent
1	7·58	10·26	9·68	0·58	76·00	24·12	19·46	4·09	11·91	9·21	1·60	10·00	6·13
2	5·76	9·01	8·24	0·77	79·00	19·91	18·90	3·95	12·82	11·20	1·76	11·00	3·85
3	4·97	11·26	9·43	1·83	91·00	20·09	14·84	3·62	12·41	11·20	1·38	8·62	4·38
4	7·26	21·48	20·16	1·32	81·00	27·61	19·12	4·76	7·16	7·36	1·45	9·06	14·88
5	7·89	6·94	5·99	0·95	95·00	11·49	10·70	4·57	11·38	11·86	1·51	9·45	1·75
6	13·58	4·72	4·22	0·50	94·00	26·10	9·40	2·36	7·69	12·67	1·72	10·76	0·70
Ls*	7·90	8·68	8·06	0·62	82·00	16·05	15·62	2·56	12·90	10·05	1·47	9·19	3·50

*Ls sample prepared in the Laboratory

B. Chemical analysis

The number of samples chemically examined are small and not much attempt will be made to draw general conclusions. Nonetheless, it could be seen from Table II that the total ash figure in sample 4 is high and is due to the abnormally high value of sodium chloride. Sample 6 shows a rather high percentage of ash insoluble in HCl as compared with the others. Samples 5 and 6 are rather low in sodium chloride. Sample 'Ls' (prepared in the laboratory) appears to be one of the best samples, as also Nos. 1 and 2, although No. 1 has a rather high sodium chloride figure.

C. *Micro-biological analysis*

Condiments that make up curry powder are bound to have a mixed flora of organisms, but it was thought that some idea should be obtained by conducting the analysis. Standard methods have been used [Tanner, 1932] and the bacterial numbers presented in Table III were as ascertained by the agar plate method (using 1·0 per cent malt agar) and also checked by counts made with Thoma's Haemacytometer. No attempt was made to identify the organisms. All the organisms belong to the aerobic group and more often than not showed pleomorphism. Fermentation of 1·0 per cent malt solution at 37°C. resulted in gas production. But when the curry powder was boiled in water for 5 min. it gave negative results (Table III). Since curry powder is usually consumed after cooking this procedure was adopted to ascertain the resistance of the organisms to boiling treatment. An interesting

TABLE III

Micro-biological analysis of curry powders

No. of sample	No. of bacteria per gram of sample		Description of bacteria	Fermentation test in 1·0 per cent Malt solution at 37°C of		Mould fragments
	No. in fresh sample	No. in sample 2 months old		Fresh sample	After boiling in water for 5 min.	
1	400,000	100,000	Short rods and coccus forms	Positive .	Negative .	Totally absent
2	310,000	110,000	Few rods and mostly coccus forms	Do. .	Do. .	Do.
3	800,000	250,000	Short rods, spore-formers	Do. .	Do. .	Few fragments seen. Number as counted by cytometer not more than 20 per gram of sample.
4	54,500	17,500	Do. . .	Do. .	Do. .	Totally absent
5	155,000	60,000	Short thick rods, profuse spore-formers	Do. .	Do.	Do.
6	320,000	100,000	Do. . .	Do. .	Do.	Do.
Ls*	160,000	75,000	Do. . .	Do. .	Do. .	Do.

*Ls sample prepared in the Laboratory

change in the initial flora was seen when bacterial counts were made after two months' storage of the curry powders (Table III). In all the samples analysed the number of viable colonies was reduced by at least two thirds. This gradual fall of the microbial numbers has been noticed in the cases of dried vegetables and fruits [Tanner, 1932] where the moisture content was below 10·0 per cent. In Table II, moisture content of all the seven samples analysed, with the exception of sample 6, is below 10·0 per cent. Curry powders and other condiments have a low moisture content and in addition present an unfavourable growth medium for microbial activity as compared with dried vegetables and fruits. It can be observed that the bacterial counts in these samples are well within the maximum standards set up for Barley malt, Rye malt and many other cereals [Tanner, 1932] and since curry powders are usually cooked with vegetables their microbial flora presents no serious difficulty and does not affect the safety of the product for human consumption.

SUMMARY

1. Seven samples of curry powders have been analysed physically (for granularity), chemically and micro-biologically.

2. A modification of the granularity test used in cereals by sedimentation of known weight of the powders in a burette containing a mixture of petroleum ether and acetone has been evolved. A fairly dependable stratification of particles of different sizes has resulted and the limitation of coarse *versus* fine particles for a good sample is given.

3. Chemical analyses have shown that percentage of total ash rises in a sample with high sodium chloride figure. Other figures for moisture, volatile extract, crude fibre, etc. have been determined in all the seven samples analysed.

4. Micro-biological analyses showed a mixed bacterial flora and the counts taken after two months' storage showed a marked fall in numbers over that present in the fresh sample. The bacterial counts recorded are well within the standard limits fixed for Barley malt, Rye malt and other cereals.

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ERRATA

For the remarks in the last column of Table III on page 636 of the December 1943 No. of the Indian Journal of Agricultural Science substitute as follows :

Methods	Year	Remarks
Sweeping	1937	L. S. S., 4F, 43F, 100F, K25
	1938	Jubilee, L. S. S., 4F, 43F, 100F, K. 25
	1939	4F, L. S. S., 100F, 43F, 124F, K. 25
	1940	4F, A. C. 31, 43F, L. S. S., 186F, K. 25, 126F
	1941	199F, 148F, 43F., L. S. S., 4F, 124F, K. 25
Counting	1937	L. S. S., 4F, 43F, 100F, K. 25
	1938	Jubilee, L. S. S., 4F, 43F, 100F, K. 25
	1939	4F, L. S. S., 100F, 43F, 124F, K. 25
	1940	4F, L. S. S., 43F, 186F, A. C. 31, 126F, K. 25
	1941	148F, 43F, 199F, L. S. S., 4F, 124F, K. 25
Fumigation	1937	L. S. S., 4F, 43F, 100F, K. 25
	1938	Jubilee, L. S. S., 4F, 43F, 100F, K. 25

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